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ORNL/TM-12029

OAK RIDGE NATIONAL LABORATORY

MARTIN MARIETTA

ARMY PROJECT ORDER NO: 89PP9921

REPORT NO: DOE IAG 1016-B123-A1

TITLE: CHARACTERIZATION OF EXPLOSIVES PROCESSING WASTE DECOMPOSITION DUE TO COMPOSTING

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Oak Ridge, Tennessee 37831-6120
managed by
Martin Marietta Energy Systems, Inc.
for the
U.S. Department of Energy
underContract DE-AC05-84OR21400

REPORT DATE: November 1, 1991

TYPE OF REPORT: Final, Phase II Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

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DOE Interagency Agreement No. 1016-B123-A1

Supported by
U. S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Project Officer: Dr. W. D. Burrows
U. S. Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010

Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831
managed by
Martin Marietta Energy Systems, Inc.
for the
U. S. Department of Energy
under contract DE-AC05-84OR21400

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1 November, 1992	Final, Phase II Report

4. TITLE AND SUBTITLE
Characterization of Explosives Processing Waste Decomposition Due to Composting.

5. FUNDING NUMBERS
Army Project Order No. 89PP9921
62720A
3E162720A835.00.005
WUDA318282

6. AUTHOR(S)
Wayne H. Griest; R.L. Tyndall; A.J. Stewart; C.-h. Ho; K.S. Ironside; J.E. Caton; W.M. Caldwell; E. Tan

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831-6120

8. PERFORMING ORGANIZATION REPORT NUMBER
DOE IAG No. 1016-B123-A1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Devleopment Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Static pile and mechanically stirred composts generated at the Umatilla Army Depot Activity in a field composting optimization study were chemically and toxicologically characterized to provide data for the evaluation of composting efficiency to decontaminate and detoxify explosives-contaminated soil. Characterization included determination of explosives and 2,4,6-trinitrotoluene metabolites in composts and their EPA Synthetic Precipitation Leaching Procedure Leachates, leachate toxicity to Ceriodaphnia Dubia and mutagenicity of the leachates and organic solvent extracts of the composts to Ames bacterial strains TA-98 and TA-100. The main conclusion from this study is that composting can effectively reduce the concentrations of explosives and bacterial mutagenicity in explosives - contaminated soil, and can reduce the aquatic toxicity of leachable compounds. Small levels of explosives and metabolites, bacterial mutagenicity, and leacable aquatic toxicity remain after composting. The ultimate fate of the biotransformed explosives, and the source(s) of residual toxicity and mutagenicity remain unknown.

14. SUBJECT TERMS
Toxicity; RAIII; Composting; Characterization; Chemistry; Decontamination

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unclassified

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Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

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EXECUTIVE SUMMARY

Static pile and mechanically stirred composts generated at the Umatilla Army Depot Activity (UMDA, Umatilla, OR) in a field composting optimization study by Roy F. Weston, Inc. were chemically and toxicologically characterized to provide data for the evaluation of composting efficiency to decontaminate explosives-contaminated soil. Static pile composts included 7, 10, 20, 30, and 40 volume % contaminated soil, with a 10% uncontaminated soil compost for a negative control, and 100% contaminated soil (not composted) for a positive control. Two mechanically stirred composts with 25 and 40% contaminated soil also were examined. All composts were sampled at the start and end of the composting period, and the uncontaminated soil and 10% soil static pile composts and the two mechanically stirred composts were sampled throughout the composting period. Characterization included determination of explosives and 2,4,6-trinitrotoluene (TNT) metabolites in the composts and their EPA Synthetic Precipitation Leaching Procedure leachates, leachate toxicity to Ceriodaphnia dubia, and mutagenicity of the leachates and organic solvent extracts of the composts to Ames bacterial strains TA-98 and TA-100.

The concentrations of explosives in the composts and their leachates, bacterial mutagenicity in the composts, and aquatic toxicity of the leachates decreased rapidly after ca. 20 days of composting. The percentage decreases observed in the final composts versus the 100% soil ranged as follows: TNT: 77.5 - 99.9%, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX): 0-97.2%, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX): 0-85.0%, specific mutagenicity with strain TA-98 (without S9 metabolic activation): 69.3-96.6%, specific mutagenicity with strain TA-100 (without S9 metabolic activation): 77.8-99.1%, toxicity of leachate to Ceriodaphnia dubia (fecundity endpoint): 45-92%. Generally, the greater the percentage of soil in the compost, the less efficient the composting was. Bacterial mutagenicity could not be determined directly in the leachates because of the large dilution from the 20:1 liquid:solid leaching ratio and interferences from bacteria in the amendments. Composting in static piles appeared most efficient through ca. 20 volume % of contaminated soil, and in the mechanical composters, through ca. 25% soil. For a given percentage of soil, the mechanical composters were more efficient than the static piles, probably because of the better aeration and mixing of the former, as well as a more active amendment mixture. The explosives and TNT metabolites determined by HPLC did not account for the observed bacterial mutagenicity. Generally less than 20% of the activity was accounted for by the compounds detected, suggesting that metabolites not detectable by HPLC (or other species) contribute the majority of the mutagenicity. Extraction and digestion of a compost inoculated with radio-labelled TNT suggested that a major portion of the biotransformed TNT was chemically bound to the compost and not mineralized.

Estimation of leachate toxicity to humans was approached by comparing the concentrations of TNT, RDX, and HMX with 100-times their EPA Drinking Water Equivalent Levels (assuming a 100-fold dilution of leachate in drinking water supplies, as in RCRA). The leachates for the most efficient composts meet these criteria, suggesting that toxicity to humans is not a serious concern.

The main conclusion from this study is that composting can effectively reduce the concentrations of explosives and bacterial mutagenicity in explosives-contaminated soil, and can reduce the aquatic toxicity of leachable compounds. Small levels of explosives and metabolites, bacterial mutagenicity, and leachable aquatic toxicity remain after composting. The ultimate fate of the biotransformed explosives, and the source(s) of residual toxicity and mutagenicity remain unknown.

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1. INTRODUCTION

Laboratory, pilot scale, and field studies (1-3) have suggested that composting can be a viable alternative to incineration for the cleanup of soils and sediments contaminated with explosives. Phase I of this project demonstrated (4) only very low aquatic toxicity, mutagenicity, and concentrations of explosives and 2,4,6-trinitrotoluene (TNT) metabolites were present in the aqueous leachates from explosives-contaminated soil which had been composted in field experiments at the Louisiana Army Ammunition Plant (LAAP). However, the results of this characterization must be considered only as preliminary for composting, because that composting study was originally designed as an engineering study, and the necessary controls for toxicology were not available. The chemical and toxicological characterization was added approximately one year after the composting had been completed.

This report describes the result of the Phase II studies. Explosives-contaminated soil at the Umatilla Army Depot Activity (UMDA) at Umatilla, OR was composted by Roy F. Weston, Inc., and the necessary controls for chemical and toxicological characterization were included from the start. The composting is described in detail elsewhere (5). Table 1. 1 lists the compost samples which were provided for this study. Three sets of composts were generated. The first was a group of static compost piles with 7, 10, 20, 30, and 40 volume percent of explosives-contaminated lagoon soil. The main variable thus was the volume % of soil in the compost. The amendment mixture was 30% sawdust, 15% apple pomace, 20% chicken manure, and 35% chopped potato waste. The negative control was a static pile compost with 10 volume % of uncontaminated soil of the same type as the contaminated soil (this will be identified as the "0% soil" compost). The positive control was noncomposted, contaminated soil ("100% soil"). The samples from these compost piles consisted of dried and homogenized composites prepared from samples collected at 5 points within the piles. Samples were provided for the start ("day 0") and finish of composting (day 90) for all static pile composts. In addition, samples were provided for the intermediate composting days 10, 20, and 44 for the 0% and 10% soil piles.

Two of the four mechanically stirred composts also were provided. These consisted of 25 and 40 volume % contaminated soil in stirred reactors (identified as MC-3 and MC-4, respectively). The amendment mixture consisting of 44% sawdust/alfalfa (50/50 mixture), 33% cow manure, 6% apple waste, and 17% chopped potato waste. This set differed from the static piles in having mechanical agitation and a different amendment mixture. The length of composting also was shorter; 44 days versus 90 days for the static composting piles. Dried and homogenized composite samples were provided for days 0, 10, 20, and 44 for the 25% soil. Similar dried and homogenized but not composited individual samples (5 each) were provided for the 40% soil composts at the same days of composting. Finally, one additional static pile compost was generated with a 10% volume of contaminated soil and the same amendments as the mechanically stirred composts. Five individual (not composited), dried and homogenized samples were received from composting days 0, 10, 20, 44, and 90.

All of the compost samples and the aqueous leachates from the US Environmental Protection Agency (EPA) Synthetic Precipitation Leaching Test (referred to as the "Clean Closure Leaching Test" or "CCLT") were characterized for explosives and TNT metabolite concentrations to determine the biotransformation efficiency of the composting and to aid interpretation of the toxicological test results. The composts or leachates from the start and finish of composting received more detailed toxicological testing because of their importance, and lesser testing was conducted on the intermediate time point samples to conserve project resources. Toxicological testing consisted of measurements of the CCLT leachate toxicity to Ceriodaphnia dubia, Ames bacterial mutagenicity of the leachates and composts (the latter as organic solvent extractable matter), and a rat oral toxicity screen. These tests were selected to gauge the toxicity of the composts and the degree of detoxification of the contaminated soil by the process of composting.

The following sections present the results of the testing. The final section integrates and summarizes the findings.

Table 1.1 Study Matrix for the Chemical and Toxicological Characterization of UMDA Composts

Compost, Vol. % Soil	Tests for Composts Sampled at Days 0	10	20	44	90
TCLP Blk	a				
(1) Static Piles:					
0	a	b	b	b	a,c
7	a				a
10	a	b	b	b	a
20	a				a
30	a				a
40	a				a,c
(2) 100% Soil	a,c				
(3) Mech. Comp.:					
25	a	b	b	a,c	
40	a	b	b	a	
(4) "New" Static Pile, 10% Soil	d	d	d	d	d

a = CCLT Leachate: *Ceriodaphnia dubia* and Ames Test, HPLC of Explosives/Metabolites,
MeCN Extracts: Ames Test, HPLC of explosives/metabolites

b = (a) without Ames Test of TCLP Leachate

c = Rat Oral Toxicity Screen

d = HPLC of explosives/metabolites

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2. PREPARATION AND CHEMICAL CHARACTERIZATION OF COMPOSTS AND LEACHATES

2.1 Source of Composts

The composts tested in this study were generated at the Umatilla Army Depot Activity (UMDA) at Umatilla, OR, by Roy F. Weston, Inc. The field composting is reported in detail elsewhere (5). Dried and homogenized aliquots of the composts were shipped to the Oak Ridge National Laboratory (ORNL), where they were stored in the dark at 4°C.

2.2 Sample Preparation

The composts were subjected to two types of preparation for this study:

(a) Aqueous leaching by the U.S. EPA Synthetic Precipitation Leaching Test (referred to as the "Clean Closure Leaching Test" or CCLT in this report). SW-846 method 1312 was followed. Briefly, the composts were leached for 18 hrs using water acidified to pH 5 with a mixture of nitric and sulfuric acids, and were pressure filtered through 0.7 μm porosity glass fiber media.

(b) Organic solvent extraction. For analyses of explosives and TNT metabolites, 1 g of compost was extracted with 4 mL of acetonitrile for 18 hrs in an ultrasonic bath with cooling, and the supernatant was recovered after the solids settled out. For Ames testing, 4 g of compost were extracted with 20 mL of acetonitrile, and 10 mL of the supernatant were evaporated to dryness in a rotary evaporator.

The CCLT models leaching of surface-applied treated wastes by acid rain. It was conducted on the composts to test the leachable toxicity of the compost products. The Toxicity Characteristic Leaching Procedure (the "TCLP") was not used here because the composted products will not be disposed in a municipal landfill. In addition, the acetate in the TCLP interferes with the toxicity tests used in this study. Composts from specific time points during composting (see Table 1.1) were leached and tested to determine changes in leachable toxicity. The tests included analysis of explosives and TNT metabolites, toxicity to Ceriodaphnia dubia, and Ames bacterial mutagenicity.

The organic solvent extraction was necessary to analyze explosives and TNT metabolites in the composts during composting. It also was necessary to add bacterial mutagenicity testing of the extracts when it was found that mutagenic activity could not be measured in the leachates. The latter apparently was a result of the large dilution from the protocol 20:1 liquid:solid leaching ratio, and

interferences from the bacteria in the leachates (see Section 4). The Ames tests of the extracts are considered as measures only of the compost mutagenicity, and not necessarily of environmentally-leachable activity.

2.3 Characterization of Leachates

Leachate characterization is presented in Tables 2.1-2.4. The pH of the CCLT leachates are listed in Table 2.1 for the static pile composts, and in Table 2.2 for the mechanically stirred composts. Whereas the contaminated soil leachate was alkaline, the pH of the day 0 compost leachates were usually acidic. The pH rose with time for both types of composting, and at the end of composting was near neutrality, as observed previously for the LAAP compost leachates (4) The leachate for the day 10 of both the 10% contaminated soil and uncontaminated soil composts were lower in pH than those of later composts. The leachates for the mechanical composters show the same increase in pH with composting time.

Table 2.1 pH of CCLT Leachates from Static Pile Composts

Sample Leached	Days of Composting	Leachate pH
Blank CCLT (no compost)	-	5.00
10% Uncontaminated Soil	0	7.05
	10	6.40
	20	7.11
	44	7.64
	90	7.68
7% Contaminated Soil	0	5.90
	90	7.83
10% Contaminated Soil	0	6.30
	10	5.10
	20	6.00
	44	7.63
	90	7.63
20% Contaminated Soil	0	7.35
	90	7.74

Table 2.1 pH of CCLT Leachates from Static Pile Composts (Continued)

Sample Leached	Days of Composting	Leachate pH
30% Contaminated Soil	0	6.70
	90	7.60
40% Contaminated Soil	0	7.20
	90	7.75
100% Contaminated Soil (not composted)	-	8.50

Table 2.2. pH of CCLT Leachates From Mechanical Composting

Compost	Days of Composting	pH of Leachate
MC-3	0	4.63
	10	7.03
	20	7.56
	44	7.64
MC-4	0	6.39
	10	7.04
	20	7.17
	44	7.20

Data for explosives and TNT metabolites in the leachates are presented in Tables 2.3 and 2.4 for the static pile and mechanical composters, respectively. These compounds were determined using the mixed mode, anion exchange/reverse phase high performance liquid chromatography (HPLC) method described in the previous report (4). This method has received a USATHAMA Level IB Certification (6). The TNT concentration in the 10% contaminated soil compost at day 0 was 35 mg/L. An initial rise in leachable TNT at 10 days of composting was evident, and may correlate with the elevated acidity of the leachate (Table 2.1). The leachability of the TNT and its solubility on the CCLT leaching fluid appear to be the limiting factors because the concentration of TNT in the composts was appreciable (see below), and the aqueous solubility of TNT is very low (100 mg/L at 25°C

in pure water, reference 7). The TNT concentration then dropped rapidly with time, and at 90 days, was 9 mg/L. A plot of the time course of TNT metabolite formation (Figure 2.1) shows that the 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT) steadily dropped while the 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT) initially rose, and then dropped as 2,4-diamino-6-nitrotoluene (2,4-DA-6-NT) and 2,6-diamino-4-nitrotoluene (2,6-DA-4-NT) slowly rose in concentration. Other TNT metabolites, such as 2,4,6-trinitrobenzoic acid, 2,4,6-trinitobenzyl alcohol, 4-hydroxyamino-2,6-dinitrotoluene, and 2,2',6,6'-tetranitro-4,4'-azoxytoluene, were not detected. The TNT metabolites present in the day 0 compost leachates undoubtedly arose from microbial action in the piles between the time of mixing and the start of the composting experiment. They also could arise during the 18 hr aqueous leaching, which was conducted at room temperature.

A bar graph comparing the concentrations of TNT and metabolites in the leachates of the static pile composts at day 90 is shown in Figure 2.2. TNT concentrations in the final leachates generally paralleled the percent soil in the compost, suggesting that as soil percent increased, the lesser percentage of amendments was less efficient in biotransforming TNT. On the basis of leachable explosives and metabolites, 30% appears to be the maximum percent of soil for a static pile with this amendment before composting efficiency drops off drastically.

Table 2.3. Explosives and TNT Metabolites in CCLT Leachates of Static Pile Composts and Soil

SAMPLE		Concentration, mg/L				
		2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	TNT	MISC
CCLT BLANK		<0.15	<1.07	<0.94	<1.17	
10% Uncontaminated Soil,	Day 0	<0.15	<1.07	<0.94	<1.17	
	Day 10	<0.15	<1.07	<0.94	<1.17	
	Day 20	<0.11	<0.12	<0.10	<0.10	
	Day 44	<0.11	<0.12	<0.10	<0.10	
	Day 90	<0.11	<0.12	<0.10	<0.10	
7% Contaminated Soil,	Day 0	<0.15	6.41	5.45	10.5	
	Day 90	≤0.24	1.51±0.80	≤0.16	4.97±0.06	HMX=3.05±0.25
10% Contaminated Soil,	Day 0	<0.15	3.38	6.51	35.0	
	Day 10	<0.15	3.91	4.92	51.5	
	Day 20	<0.38	5.86±0.47	5.94±0.48	32.5±2.92	
	Day 44	5.06±0.07	1.68±0.11	<0.29	12.4±1.83	HMX=3.70±0.13
	Day 90	2.43±0.06	1.43±0.05	<0.20	9.07±0.13	HMX=3.47±0.16

Table 2.3. Explosives and TNT Metabolites in CCLT Leachates of Static Pile Composts and Soil (Continued)

SAMPLE	Concentration, mg/L				
	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	TNT	MISC
20% Contaminated Soil, Day 0	<0.15	6.39	4.80	56.1	
Day 90	0.84 ± 0.06	6.19 ± 0.14	1.59 ± 0.02	15.4 ± 0.13	HMX= 3.98 ± 0.26
30% Contaminated Soil, Day 0	<0.33	3.23	4.16	69.2	
Day 90	<0.38	4.04 ± 0.03	1.67 ± 0.06	16.2 ± 0.06	
40% Contaminated Soil Day 0	<0.15	1.81	4.29	92.5	
Day 90	<0.77	5.26 ± 0.16	6.75 ± 0.23	68.3 ± 0.98	
100% Contaminated Soil (Not Composted)	<0.57	<1.53	<1.63	72.0	

Notes: "<" indicates "not detected": Differences among "<" for given constituent reflects different dilutions before HPLC. Std. dev. shown for samples analyzed in 3 replicates. RDX could not be analyzed because of chromatographic interference. 2,6-DA-4-NT and other TNT metabolites were not detected in any sample. HMX was detected in some samples (as noted) at low dilution, but was below reporting limit, and data are considered as estimates.

Table 2.4. Explosives and TNT Metabolites in CCLT Leachates of Mechanically Stirred Composts.

Compost Leached	Concentration in Leachate, Avg. ± Std. Dev., mg/L (n=3[a])						
	2,6-DA-4-NT	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	2,4,6-TNT	RDX	HMX
MC-3, 25% Soil, Day 0	<3.8	<5.3	3.2±0.46[b]	≤3.0±0.75[b]	60±0.75	14.4±0.35	<21
MC-3, 25% Soil, Day 10	<3.8	≤4.4±1.6[b]	<9.0	5.7±0.52[b]	<3.0	8.3±0.84	<21
MC-3, 25% Soil, Day 20	≤1.1±0.07	0.82±0.07[b]	<2.6	3.7±0.01	<0.8	7.3±0.10	4.4±0.25[b]
MC-3, 25% Soil, Day 44	<0.75	<1.1	<1.8	[illegible]	<0.6	≤1.3±0.21	2.5±0.22[b]
MC-4, 40% Soil, Day 0	<3.8	<5.3	<9.0	<3.8	67.4±3.8	14.3±0.38	<21
MC-4, 40% Soil, Day 10	<3.8	<5.3	<9.0	[illegible]	83.2±1.2	17.7±0.62	<21
MC-4, 40% Soil, Day 20	<3.8	<5.3	5.8±0.53[b]	[illegible]	34.2±0.4	18.2±0.69	<21
MC-4, 40% Soil, Day 44	<2.5	<3.5	3.1±0.12[b]	[illegible]	<3.0	17.1±0.92	<14

[a]Average ± standard deviation for three analyses of a single leachate. "≤" indicates that no compound was detected for one of the replicates, and the reporting limit was used in the calculation.
[b]Includes concentrations measured below the reporting limit, and are considered as estimates.

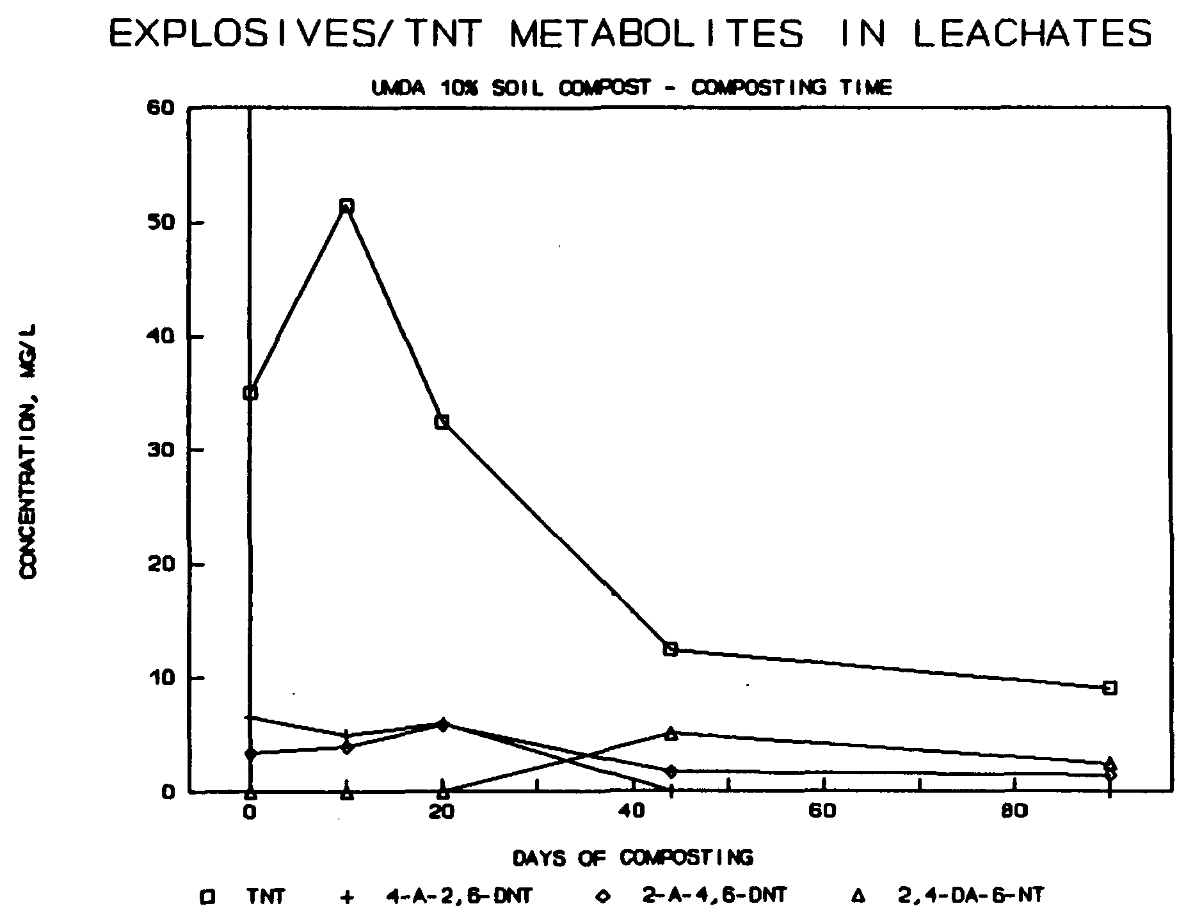


Figure 2.1 Concentrations of TNT and Metabolites in Leachates of 10% Soil Static Compost as a Function of Composting Time.

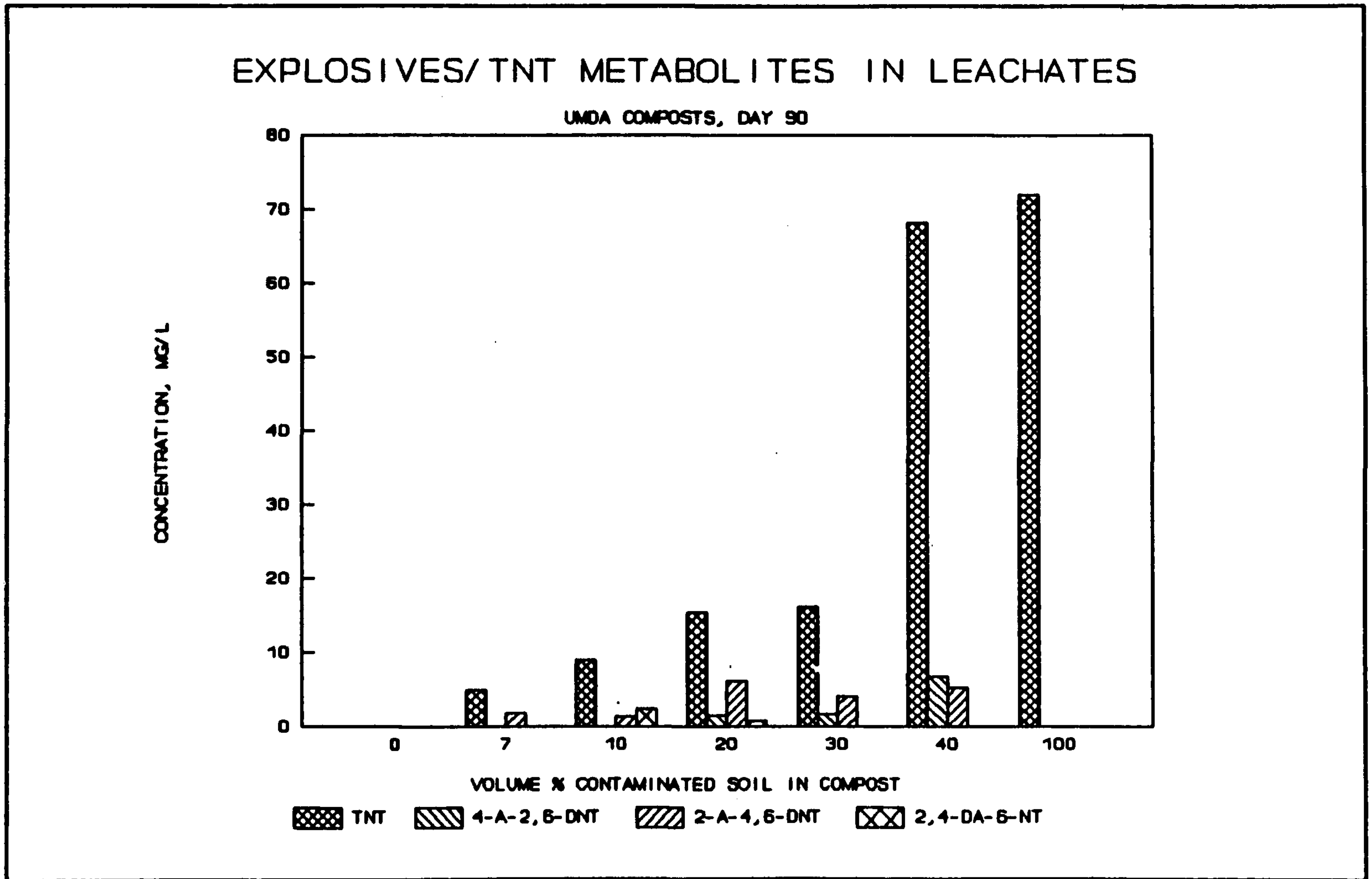


Figure 2.2 Comparison of TNT and Metabolites in Leachates of Static Pile Composts at Day 90.

The data in Tables 2.3 and 2.4 show that the mechanical composters were able to more rapidly transform the leachable explosives, and that for a given percentage of soil, the mechanical composter was more efficient than the static pile compost. However, different amendments were used for the two types of composting, and as will be discussed below, the amendment also had a major influence upon biotransformation.

2.4 Characterization of Composts

An extraction study examined the recoveries of the explosives and TNT metabolites, and a carbon-14 ring-labelled 2,4,6-trinitrotoluene (^{14}C-TNT) tracer. The latter was to be used in the analysis of the composts to monitor explosives/metabolites recoveries, and the relationships among their recoveries needed to be tested. USATHAMA Standard Soil was spiked at 10-fold the detection limit (n=6) and at the detection limit (n=1) with explosives and TNT metabolites and with a concentration of ^{14}C-TNT which was not detectable by HPLC, but which could be determined readily using liquid scintillation counting. The samples were extracted and analyzed using a method which passed THAMA Level IB certification. Briefly, 1 g of soil was extracted in an ultrasonic bath for 18 hrs at room temperature with 4 mL of acetonitrile. The supernatant was diluted with water and analyzed using the mixed mode anion exchange/reverse phase HPLC method described previously (4), following THAMA IB QC. The results of this study (Table 2.5) showed good recoveries and precision for all the analytes at 10 times the detection limit. Two aliquots had unusually high results for TNT, and after their elimination, the TNT results were in line with the rest of the data. At the detection limit, only HMX yielded a low recovery. The sensitivity for HMX is the lowest of the set. The radiotracer appears to model the recovery of the explosives, but the range of recoveries was limited with this sample matrix.

Data from the analysis of explosives and TNT metabolites in the static pile composts are listed in Table 2.6, and for the mechanical composters and the "new" static pile 7 in Table 2.7. As observed for the leachates, the greater the percentage of soil in the compost, the less the biotransformation of the explosives. The greater volume of soil decreased the volume of amendments available to enhance biotransformation. For equivalent percentages of soil, the mechanical composters were more rapid and efficient than the static piles, probably because of their greater aeration and more uniform mixing. However, the amendments also were different between the static piles and the mechanical composters, and thus at least two variables were changed between the two series of experiments. For both types of composting, the biotransformation was greatest for TNT, followed by RDX, and then HMX. The maximum soil percentage for static piles before efficiency dropped off was about 30%. This is evident in the bar graphs plotted in Figure 2.3.

The amendment also appeared to have an important effect upon biotransformation efficiency. The "new" stack 7 (10% soil, Table 2.7) was much more efficient in explosives transformation than was the old stack (Table 2.6). In addition to an efficient TNT

transformation, it also achieved by day 90 the lowest RDX and HMX concentrations of any of the composts tested.

The concentration of TNT in the static pile compost (Figure 2.4) dropped with time of composting, while the 4-A-2,6-DNT initially rose and then fell, while the 2-A-4,6-DNT dropped steadily and the diamino metabolites rose. In the earlier static pile

Table 2.5. Comparison of Recoveries for Explosives, TNT Metabolites, and Carbon-14 Labeled TNT in Spike Recovery Study Using THAMA Standard Soil.

Compound	Recovery, %	
	10 X DL Spike[a] Avg. ± Std. Dev.	DL Spike[b], Avg.
2,6-DA-4-NT	97 ± 5.4	84
2,4-DA-6-NT	90 ± 5.2	83
2-A-4,6-DNT	102 ± 5.4	105
4-A-2,6-DNT	103 ± 6.3	71
1,3,5-TNB	108 ± 8.5	153
2,4,6-TNT	126 ± 44 (98 ± 2.0[c])	102
RDX	103 ± 11	99
HMX	103 ± 9.6	41
^{14}C-TNT[d]	92 ± 3.1	101

[a]Spiked at 10X detection limit, n=6.
[b]Spiked at the detection limit, n=1.
[c]Result recalculated after dropping the 2 highest results (212 and 154%), n=4.
[d]Recovery of carbon-14 labeled TNT tracer (0.2 mg/Kg) determined using liquid scintillation counting.

Table 2.6. Explosives and TNT Metabolites Analyses of Static Pile Composts.

Sample	Concentration[a], Average ± Standard Deviation, mg/Kg							Recovery[b] Avg. ± Std. Dev., %
	2,6-DA-4-NT	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	2,4,6-TNT	RDX	HMX	^{14}C-TNT
Uncont. Soil, Day 0	<3.9	<9.9	<5.1	<3.0	4.4 ± 6.2	1.9 ± 2.7[c]	<27	92 ± 4.3
Uncont. Soil, Day 10	<3.9	<9.9	<5.1	<3.0	8.2 ± 0.7	<6.7	<27	99 ± 0.7
Uncont. Soil, Day 20	<3.9	<9.9	<5.1	<3.0	1.4 ± 0.2[c]	<6.7	<27	96 ± 1
Uncont. Soil, Day 44	<3.9	<9.9	<5.1	<3.0	<2.1	<6.7	<27	93 ± 0.7
Uncont. Soil, Day 90	<3.9	<9.9	<5.1	<3.0	4.3 ± 0.2	<6.7	<27	98 ± 0.4
7% Cont. Soil, Day 0	<20	<50	494 ± 26	169 ± 14	1240 ± 144	762 ± 32	220 ± 17	90 ± 1.3
7% Cont. Soil, Day 90	2.5 ± 0.3[c]	3.9 ±0.2[c]	39 ± 17	125 ± 69	279 ± 248[d]	256 ± 45	136 ± 23	96 ± 1.6
10% Cont. Soil, Day 0	<39	<99	278 ± 17	58 ± 2.6	4630 ± 478	909 ± 38	203 ± 66[c]	95 ± 0.7
10% Cont. Soil, Day 10	<20	<50	157 ± 7.9	64 ± 2.2	1690 ± 119	799 ± 29	226 ± 17	97 ± 0.7
10% Cont. Soil, Day 20	<13	<33	175 ± 17	155 ± 13	798 ± 39	687 ± 23	193 ± 16	99 ± 2.1
10% Cont. Soil, Day 44	8.3 ± 1.5[c]	35 ± 8.4	142 ± 33	337 ± 26	197 ± 42	761 ± 30	275 ± 29	93 ± 2.9
10% Cont. Soil, Day 90	6.7 ± 0.2	69 ± 4.9	32 ± 99	110 ± 3.4	97 ± 44	395 ± 9.6	153 ± 62	94 ± 1.2

[a]N=3. "<" means compound not detected at all. Reporting Limit listed varies with sample extract dilution.
[b]Extraction recovery of carbon-14 labelled TNT determined using liquid scintillation counting.
[c]Result listed is less than reporting limit, and is an estimate.
[d]Result is 104 if one value (630) is dropped, n=2.

Table 2.6. Explosives and TNT Metabolites Analyses of Static Pile Composts. (continued)

Compost Sample	Concentration[a], Average ± Standard Deviation, μg/g							Recovery[b] % Avg. ± Std. Dev.
	2,6-DA-4-NT	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	2,4,6-TNT	RDX	HMX	^{14}C-TNT
20% Contaminated Soil, Day 0	<39	<99	355 ± 45	133 ± 13	6550 ± 363	1100 ± 77	320 ± 24	92 ± 0.5
20% Contaminated Soil, Day 90	6.5 ± 0.8[c]	21 ± 2.6[c]	92 ± 7.0	295 ± 29	143 ± 19	647 ± 11	241 ± 1.5	96 ± 1.6
30% Contaminated Soil, Day 0	<39	<99	164 ± 10	32 ± 3.0	7950 ± 199	1030 ± 43	296 ± 16	92 ± 3.2
30% Contaminated Soil, Day 90	6.5 ± 0.4[c]	90 ± 1.3[c]	132 ± 18	232 ± 27	222 ± 33	778 ± 44	319 ± 12	92 ± 1.1
40% Contaminated Soil, Day 0	<39	<99	165 ± 11	34 ± 6.2	9410 ± 712	1240 ± 52	340 ± 19	90 ± 1.2
40% Contaminated Soil, Day 90	<26	<66	322 ± 5.4	156 ± 1.5	2750 ± 135	1440 ± 120	376 ± 3.4	91 ± 4.2
100% Contaminated Soil (not composted)	<65	<165	<65	<50	12200 ± 1400	1380 ± 128	409 ± 32	84 ± 6.1
THAMA Std. Soil Blank	<3.9	<9.9	<5.1	<3.0	<2.1	<6.7	<27	99 ± 1

[a]Three replicates from a homogenized composite of individual samples collected at 5 locations in the compost piles. "<" means compound not detected at all. Reporting Limit listed. Varies with sample extract dilution.
[b]Extraction recovery of carbon-14 labelled TNT determined using liquid scintillation counting. [c]Result listed is less than reporting limit, and is an estimate. [d]Result is 104 if one value (630) is dropped, n=2.

Table 2.7. Determination of Explosives and TNT Metabolites in Mechanical Composter and New Static Pile Composts.

Compost	Concentration in Compost, Avg. ± Std. Dev., mg/Kg (n=5 except where indicated[a])						
	2,6-DA-4-NT	2,4-DA-6-NT	2-A-4,6-DNT	4-A-2,6-DNT	2,4,6-TNT	RDX	HMX
MC-3, 25% Soil, Day 0[b]	<500	<700	<1,200	<500	4,210±220	<800	<2,800
MC-3, 25% Soil, Day 10[b]	<330	<470	<800	<330	680±67	<530	<1,900
MC-3, 25% Soil, Day 20[b]	<200	<280	<480	290±37	<160	≤330±23	<1,100
MC-3, 25% Soil, Day 44[b]	<10	<14	<24	29±3.0	<8	39±3.9	102±7.9
MC-4, 40% Soil, Day 0	<500	<700	105±60.6	79.3±14.2	6,950±190	754±43.6	456±19.5
MC-4, 40% Soil, Day 10[c]	<500	<700	277±68.9	295±67.4	5,100±760	843±580	522±48.0
MC-4, 40% Soil, Day 20	<330	<470	483±59.6	558±89.6	1,790±536	840±148	627±37.3
MC-4, 40% Soil, Day 44[c]	<200	<280	323±53.4	547±57.1	209±188	621±114	601±78.7
Stack 7, 10% Soil, Day 0[c]	<500	<700	145±32.5	59.1±38.1	3,850±650	618±99.6	307±67.4
Stack 7, 10% Soil, Day 10	<200	<280	119±40.4	115±43.7	1,080±536	386±95.8	203±52.2
Stack 7, 10% Soil, Day 20	<50	<70	25±18.0	50.7±24.2	117±104	112±53.8	91.6±49.8
Stack 7, 10% Soil, Day 44	<33	<47	1.5±1.0	16.4±4.0	39.2±29.8	42.9±31.8	55.1±25.8
Stack 7, 10% Soil, Day 90	<10	<14	5.3±1.6	18.2±6.9	40.7±31.0	46.3±15.3	61.2±26.2

[a]Non-composited (but homogenized) samples taken from 5 sampling locations in the composts.

[b]Three replicates taken from one homogenized composite of 5 individual samples taken as in footnote (a).

[c]Four samples analyzed. One sample of the 5 was received broken.

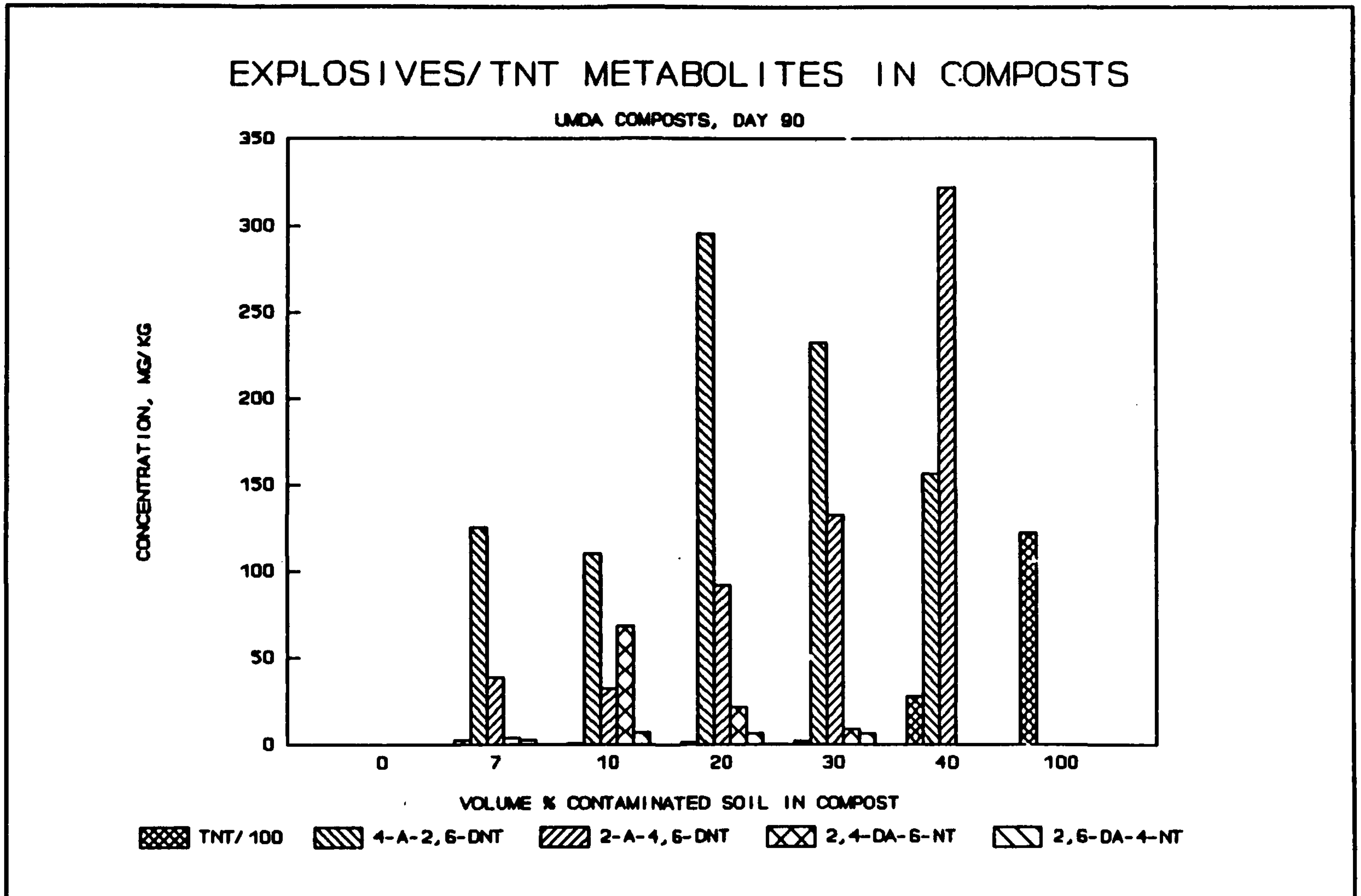


Figure 2-3 Comparison of TNT and Metabolites in Final Static Pile Composts.

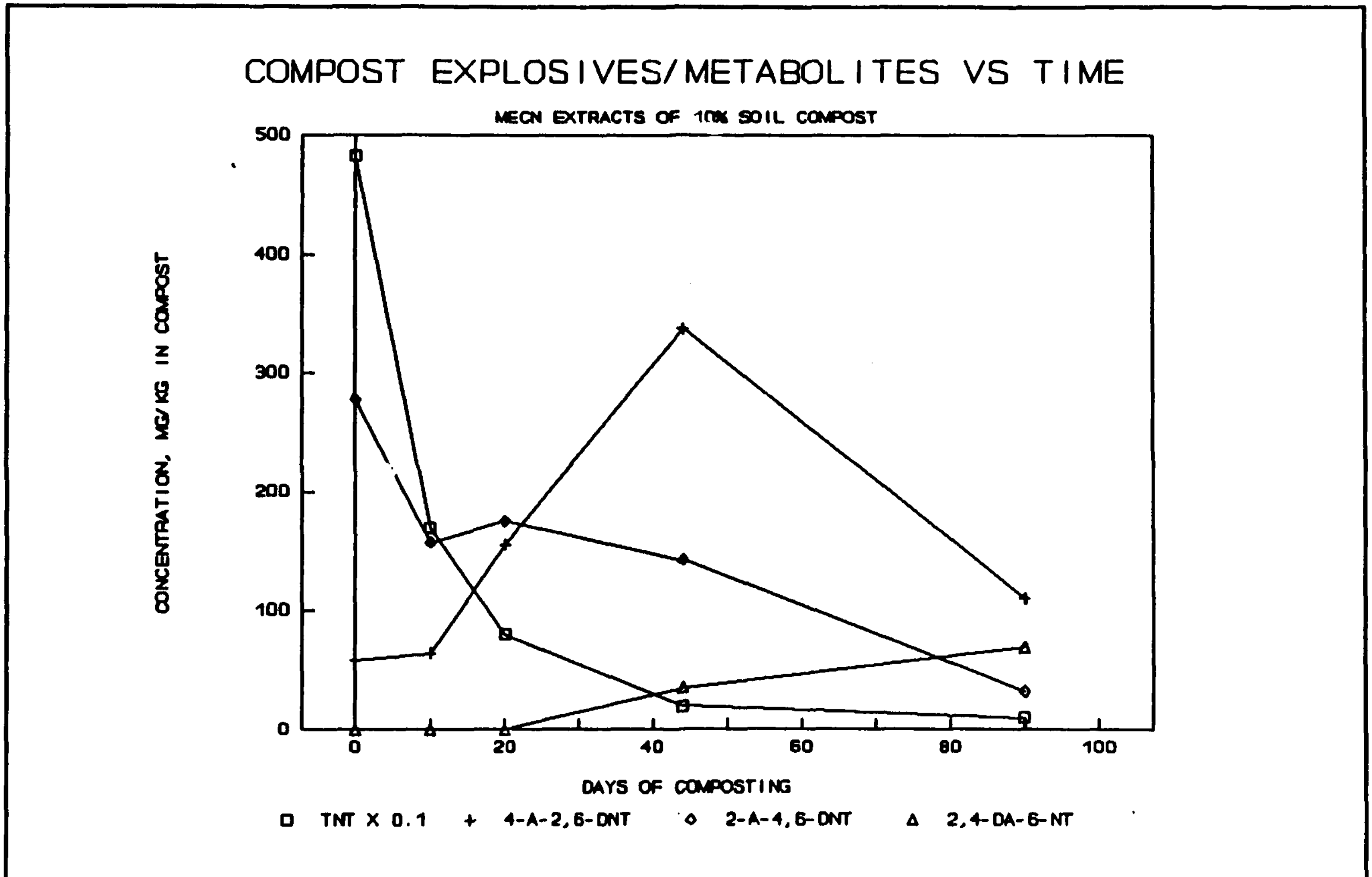


Figure 2.4 Comparison of TNT and Metabolites in 10% Soil Static Compost as a Function of Composting Time.

composting at LAAP, the concentrations of two monoamino and the two diamino TNT metabolites (5) all initially rose and then fell with composting time. The differences in results from those of this study probably reflect the much longer composting period and the lower percentage of soil (3%) in that study. It also should be noted that the differences between the relative concentrations of the explosives/metabolites in the composts and their leachates suggest that some biotransformation does indeed occur during the CCLT leaching process.

2.5 Comparison of Composting Efficiencies

The relative efficiencies for the types of composting and percentages of soil composted are evaluated in Table 2.8, which expresses the percent decrease in explosives concentrations in the material which would be returned to the field (i.e., the final composts at day 90 for static piles and day 44 for the mechanical composters) versus the 100% contaminated soil which was removed from the lagoon for treatment. Percentage decreases and their 95% confidence intervals were calculated, and those data for a particular explosive which are the same for a 5% significance level are shaded. Raw data and statistics are included in Appendix C.

Very high TNT biotransformation efficiencies (ca. 98% and greater) were achieved for all of the composts, except for the 40% static pile. For RDX, the 25% mechanical composter (MC-3) and the "new" 10% static pile were maximally efficient (ca. 97% reduction in RDX concentration). The "old" static piles were less efficient as a group, and the 7% and 10% static piles achieved the same efficiencies (but lower as a group than the 25% mechanical and "new" 10% static pile). For HMX, the 25% mechanical composter, the "new" 10% static pile, and the 7% static pile were the most efficient. The next most efficient group overlapped the first: the 7%, 10%, and 20% static piles were the same in their efficiencies. The choice of optimum composting conditions would depend of the explosives to be removed and the relative costs of the composting operations. It appears that the "new" 10% static pile and the 25% mechanical composter were most efficient overall, followed by the 7% and 10% static piles.

Chemical characterization will be compared with toxicity in the final summary section of this report.

2.6 Fate of Biotransformed Explosives

The ultimate fate of the TNT biotransformed in the composts is not clear at the present. Previous studies (1,8-10) suggest that only a small percent of the TNT is actually mineralized, and that a significant percentage can be covalently attached to

macromolecular constituents in the compost, i.e. held in an inaccessible "bound" fraction. In Table 2.9, the percentage of the TNT and metabolites in the day 0 composts which is accounted for by the metabolites and untransformed TNT in the day 10, 20, 44, or 90 compost is presented. Two trends are evident: (a) with increasing time of composting, a decreasing percentage is accounted, (b) with greater % soil in the composts, a lesser % is accounted. It appears that the final product(s) of TNT biotransformation are not determined by the analytical method. They could represent mineralization of the TNT, formation of nonextractable "bound" products, or formation of products which are extractable, but not detectable by the HPLC at the three wavelengths monitored (280, 254, and 230 nm). The first two possibilities seem most likely.

Study of the composted soil inoculated with ^{14}C-TNT provided some insight into the ultimate fate of TNT. A sample of contaminated soil was inoculated by Roy F. Weston, Inc. with 90 microcuries of ring-^{14}C-TNT. The inoculated soil was mixed with the cow manure-based amendments to form 200g of compost and split into two portions, one of which was refrigerated ("day 0" sample), and the other was placed into the new 10% soil compost pile for 90 days ("day 90" sample). The samples were shipped to ORNL for analysis. Three 1.2-1.8g aliquots of each sample were first extracted for 24 hrs with 5 mL of acetonitrile in a cooled ultrasonic bath. The extractions were repeated with fresh solvent for a total of 4 extractions to remove free TNT and metabolite. Particle-bound ^{14}C-activity in the extracts was estimated by liquid scintillation counting portions of the extracts before and after filtering through 0.45μm filters. Next, the residues were digested a total of 8 times, each with 5 mL of fresh 10% potassium hydroxide in ethanol to liberate "bound" ^{14}C-activity. The digests were heated to 60°C for 2 hrs in a heating block, and then were allowed to set in the block for 24 hrs without heat applied. The extracts and digests were filtered, and the ^{14}C-activity in each was determined by liquid scintillation counting. The extracted and digested compost residues were then sent to Roy F. Weston, Inc. for combustion and collection and liquid scintillation counting of non-hydrolyzeable "bound" ^{14}C-activity.

The results of the counting are presented in Table 2.10 as recoveries of the ^{14}C-activity inoculated in the soil. Two observations are important. First, the bulk of the inoculated ^{14}C-TNT was tied up in a bound fraction which required exhaustive alkaline digestion for liberation. This suggests (but does not prove) that it would not be readily available for environmental release. The second observation is that the bound fraction was formed rapidly (day 0), which may be an artefact. Externally inoculated TNT may be more "available" for reaction with the amendment bacteria than the native TNT, and could be biotransformed more rapidly. Although the inoculated TNT reacted more quickly than the native TNT, the results suggest that a portion of the "unaccounted" TNT in the composts is present in a bound form. Clearly more work is needed to establish TNT fate.

Table 2.8. Decrease in Explosives Concentrations of Contaminated Soil Calculated as the Percent Decrease in the Final Composts Versus 100% Contaminated Soil. (For each column, the shaded area encloses data for the highest percent decrease which are statistically the same at a 5% significance level. The next group is underlined in bold.)

Compost[a]	% Decrease in Explosives Conc.[b]		
	TNT	RDX	HMX
40% MC	98.3	55.2	0
25% MC	99.9	97.2	75.0
10% NS	99.7	96.7	85.0
7% S	97.7	**81.5**	**66.9**
10% S	99.2	**71.5**	**62.5**
20% S	98.8	53.2	**41.1**
30% S	98.2	43.8	22.1
40% S	77.5	0	8.2
0% S[c]	NA	NA	NA

[a] Volume % contaminated soil in mechanical composter (MC) or static pile (S). NS refers to "new" static pile.

[b] Percent decrease in concentrations of explosives. Shaded areas for an explosive enclose % decreases which are the same within a 5% significance level.

[c] Explosives not detected in compost of uncontaminated soil; decreases relative to 100% are not applicable.

Table 2.9. Accounting by Composting Day for the TNT and Metabolites Present in the Day 0 Compost.

Compost	Initial TNT and Metabolites Accounted for by Composting Day[a], %			
	10	20	44	90
7%, Static				15
10%, Static	37	23	16	7.0
20%, Static				8.8
30%, Static				8.1
40%, Static				34
New 10%, St.	33	5.0	1.5	1.7
25%, Stirred	<32	<17	<1.1	
40%, Stirred	80	42	17	

[a]Blank spaces indicate samples not scheduled for analysis. "<" denotes where reporting limit used in calculations.

Table 2.10. Distribution of ^{14}C-Activity in Compost Inoculated with C^{14}-TNT. (Avg ± Std. Dev. for n=3)

	% ^{14}C Accounted	
Fraction	Day 0	Day 90
"Free" (MeCN Extract)	26.2 ± 1.6	1.2. ± 0.2
"Bound" (Particle-Associated)	14.2 ± 6.7	17.9 ± 4.0
"Bound Hydrolyzeable" (KOH/ETOH Digest)	59.6 ± 2.7	56.8 ± 5.2
"Bound Non-Hydrolyzealbe" (Combustion)	3.5 ± 0.4	4.7 ± 0.2
Total	103.5	80.6

3. CERIODAPHNIA DUBIA TOXICITY TESTS OF LEACHATES

Ceriodaphnia dubia is a small freshwater crustacean commonly found in ponds and lakes in temperate regions. In 1984, the Environmental Protection Agency (EPA) developed a 7-d bioassay procedure that uses Ceriodaphnia to estimate acute and chronic toxicity of effluents and receiving waters (11). These methods are now available as standard operating procedures (12) and are used frequently for both effluent and ambient toxicity assessments (13,14). Ceriodaphnia are 1.5 to 2 mm in size when mature, are more sensitive than fish to many toxicants (15), parthenogenic (16), reach maturity in three to four days, rarely live longer than about 40 d, and produce many offspring [they typically produce 8 to 12 broods, each containing 3 to 18 offspring; (12)]. Collectively, such features make Ceriodaphnia especially well suited for water-quality assessments.

The objective of this portion of the study was to determine the efficacy of composting as a means to lower the toxicity of soils contaminated with explosives such as TNT, RDX and HMX. To meet this objective, Ceriodaphnia 7-d tests were conducted to estimate the toxicity of CCLT leachates prepared from soil that had been contaminated with TNT, to various degrees, before being composted, for various durations, in static piles or mechanically-stirred reactors.

3.1 Materials and Methods

Dilutions of each CCLT leachate to be tested were prepared by adding leachate to an appropriate volume of diluted mineral water (Perrier; diluted to 20% of full-strength with deionized distilled water). Each dilution of each leachate was then tested with Ceriodaphnia (10 replicates, each containing 15 mL of test solution and one neonate). In each temporal block of tests, Ceriodaphnia survival and reproduction was also evaluated through the use of a reference, which consisted of a set of 10 replicates containing just diluted mineral water (one neonate per replicate). This reference validated the biological quality of the dilution water, the Ceriodaphnia food, the test conditions (e.g., incubation temperature and photoperiod), and the health of the neonates used to initiate the tests.

Information about the leachates, including the concentration of contaminated soil in the compost, the duration of composting, the type of composting procedure (static pile versus mechanically stirred), and the date that the leachate was tested for toxicity, is summarized in Appendix D.

Within each temporal block of tests, a leachate's toxicity was determined by comparing survival and reproduction of Ceriodaphnia among the concentrations tested. In most instances, the survival and reproductive responses of the Ceriodaphnia differed strongly among leachate concentrations and generated conspicuous dose-response curves. The concentration of leachate reducing survival by 50% (the LC_{50}) was then determined

graphically by interpolation. We computed the concentration of leachate needed to reduce reproduction of Ceriodaphnia by 50% (the EC_{50}) and also to 15 offspring per female and expressed that latter concentration in terms of toxicity units (TUs). TUs were computed by taking the reciprocal of the concentration (in percentage) needed to lower reproduction to 15 offspring per female. Fifteen offspring per female was selected as the "standard" point for comparing leachate effects because this value was consistently lower than controls, well above zero, and is the minimum level of fecundity acceptable for valid controls according to EPA protocol [see (12)]. In some instances, the highest tested concentration of a leachate was not great enough to reduce either survival or reproduction by 50%. When this occurred, a new leachate was prepared and tested at higher concentrations.

3.2 Results

Leachate toxicity to Ceriodaphnia dubia is summarized in Table 3.1. The endpoint data for survival (as the LC_{50}) and fecundity are listed. For fecundity, both the conventional EC_{50} and an SR_{15} (the concentration at which the number of offspring per female is 15) have been calculated. The full set of data is included in Appendix D.

Reductions in Ceriodaphnia survival are generally indicative of acute toxicity, while reductions in fecundity are used as evidence for chronic toxicity. These generalities were supported strongly by the results of the tests reported here. In almost every instance, Ceriodaphnia fecundity was reduced at a leachate concentration that was lower than that needed to cause a significant reduction in survival.

An important finding from the toxicity testing component of the study was the time-dependent reduction in acute and chronic toxicity of the leachates. The pattern of "longer composting -- lower toxicity" was evident for leachates of composts both from the static piles and the mechanically-stirred reactors (Table 3.1). The benefits of longer composting periods were especially evident in the MC-3 (25% contaminated) series of samples. In this group, for example, compost day zero leachate was acutely toxic at a 5% concentration. After 44 d of composting, though, even the 20% concentration of the leachate lowered reproduction by less than 50% (Table 3.1). Leachate toxicity declined slightly faster in the MC-3 series of composts than it did in the MC-4 series. For the 10%-contaminated compost, there was a 10- to 15-fold loss in chronic toxicity of the leachates over the 90-d composting period (Fig. 3.1).

Another important finding from the toxicity testing was that the extent of compost contamination was an important determinant of toxicity after even an extended period of composting. Static composting, for example, was used in an attempt to lower the TNT content (and toxicity) of 7%, 10%, 20%, 30% and 40% concentrations of contaminated soil. The leachates from this composting series showed a clear trend of "more contamination -- greater toxicity" even after 90 d of composting (Fig. 3.2). Thus, lower

concentrations of explosives, and a longer composting duration, were both important determinants in lowering the toxicity of the leachates in the composting experiments.

3.3 Discussion

Naturally occurring soil- and sediment-dwelling microbes produce a diverse array of exo- and endoenzymes that can degrade even recalcitrant and toxic organic compounds. The rate at which such degradation occurs can be fast if (a) initial concentrations of the material are not great enough to inhibit the degradation process, and (b) conditions favorable to the biota involved with the degradation, including temperature, pH, adequate supply rates of appropriate electron acceptors and carbon substrates, etc. are maintained. Explosives such as TNT contain energy-rich chemical bonds between carbon and nitrogen. Such bonds should be particularly vulnerable to attack by consortia of soil microbiota: nitrogen is often the limiting nutrient in northern temperate forest ecosystems and grasslands (cf. 17,18), and organic carbon serves as the primary source of electrons required to support most heterotrophic microorganisms (19). The results of this study show that TNT can be degraded, through composting operations, by consortia of microbes. Additionally, the loss of TNT by microbial processes was accompanied by commensurate reductions in compost leachate toxicity and mutagenicity. Thus, biotechnological approaches for lowering TNT concentrations and adverse biological effects of this contaminant seem viable.

Anaerobic liquid-phase bioreactors are now commonly used to destroy constituents such as nitrates and sulfates; diverse organic wastes, too, are commonly treated by aerobic liquid-phase digestors. The efficacy of solid-phase bioreactors, wherein sediments or soils contaminated with organics are decontaminated through the use of microbes, has been far less well documented. The elimination or reduction of TNT in sediment or soil by composting serves as an excellent example of the application of solid-phase biotechnology in waste management and remediation.

Several aspects of composting as a means to eliminate TNT from solid phase may need to be considered in more detail. Clearly, the viability of the composting option depends in part upon its cost relative to alternative procedures, such as combustion. The cost of composting will be affected by the kinds of amendments that may be required, plus the need for manpower and/or equipment to consolidate the contaminated soil or sediment, mix it with the whatever amendments are necessary, and periodically stir or mix the compost to ensure homogeneous and near-total degradation. Analyses required to demonstrate efficacy and biological acceptability of residues from the composting procedure are also required. This study shows that both chemical measurements of TNT and biological measurements of the toxicity of compost leachate can be used to verify the efficacy of composting for detoxifying soil or sediment contaminated with TNT. The EPA procedure for testing toxicity of ambient or effluent samples with Ceriodaphnia proved

useful in this regard: these organisms were sensitive to the presence of the contaminants in the compost samples and data from such tests can be available for management or regulatory decision purposes rapidly (i.e., 7-8 d) after the compost leachates have been prepared.

The efficacy of composting is likely to vary with climatic conditions, soil type, and biotic factors such as the presence of appropriate assemblages of microorganisms. A field test, wherein one type of TNT-contaminated soil or sediment was sent to various geographic locations selected to encompass a specific range in environmental conditions could provide much information about the potential for using composting to decontaminate sediments or soils at munitions facilities across the U.S.

A final consideration could be an assessment of the long term suitability of the composted wastes for land application. Presumably, the fully-composted final residue from a composting operation would be applied to a terrestrial habitat. There, it would become integrated into the soil by plants, soil bacteria and fungi, micro- and macroinvertebrates (e.g., arthropods, earthworms) and small burrowing mammals, such as shrews, voles, mice, moles, etc. It is possible that sustained exposure to low concentrations of explosives degradation products could adversely affect sensitive physiological processes, such as reproduction, of some animals or plants. Although unlikely, only a well-designed field study could be used to definitively negate the presumption of ecological risk.

Table 3.1. Comparison of Ceriodaphnia dubia Data for Various Endpoints with CCLT Leachates of UMDA Composts

CCLT		Leachate Concentration (%)		
Leachate of Compost	Composting Days	LC_{50}	EC_{50}	SR_{15}
Blank	-	>90	>90	>90
Non Cont.	0	>20	17	17.9
	10	16.7	6.1	5.7
	20	>20	3.0	2.2
	44	>50	>50	>50
	90	>50	43	>50
7% Soil	0	10	<5	<5
	90	>50	31	34.5
10% Soil	0	1.3	0.5	<0.5
	10	5.8	0.7	0.6
	20	6.4	<0.5	<0.5
	44	8.3	2.0	2.3
	90	18	7.2	7.2
20% Soil	0	4	<1	<1
	90	>20	8.4	8.1
30% Soil	0	4	1	1.1
	90	>50	21	19.5
40% Soil	0	4	1	1.3
	90	15	4.6	4.6
100% Soil	-	>5	2.5	2.4
MC-10	0	5	0.8	0.8
LAAP Meso.	-	90	44	-
LAAP Therm.	-	>100	80	-

Table 3.1. Comparison of Ceriodaphnia dubia Data for Various Endpoints with CCLT Leachates of UMDA Composts (Continued)

CCLT		Leachate Concentration (%)		
Leachate of Compost	Composting Days	LC_{50}	EC_{50}	SR_{15}
UMDA MC-4	0	3.8	1.2	1.7
	10	3.8	1.4	1.9
	20	7.5	<1	<1
UMDA MC-4	44	>20	9.2	8.5
MC-3	0	3.9	<0.5	<0.5
	10	11.5	2.5	2.2
	20	<20	6.6	6.3
	44	<20	20.3	18

Fig. 3.1. Effect of composting duration on toxicity of leachates from 10%-contaminated compost. A toxicity unit (vertical axis) is the reciprocal of the concentration of a leachate, expressed as a percentage, needed to reduce Ceriodaphnia reproduction to 15 offspring per female.

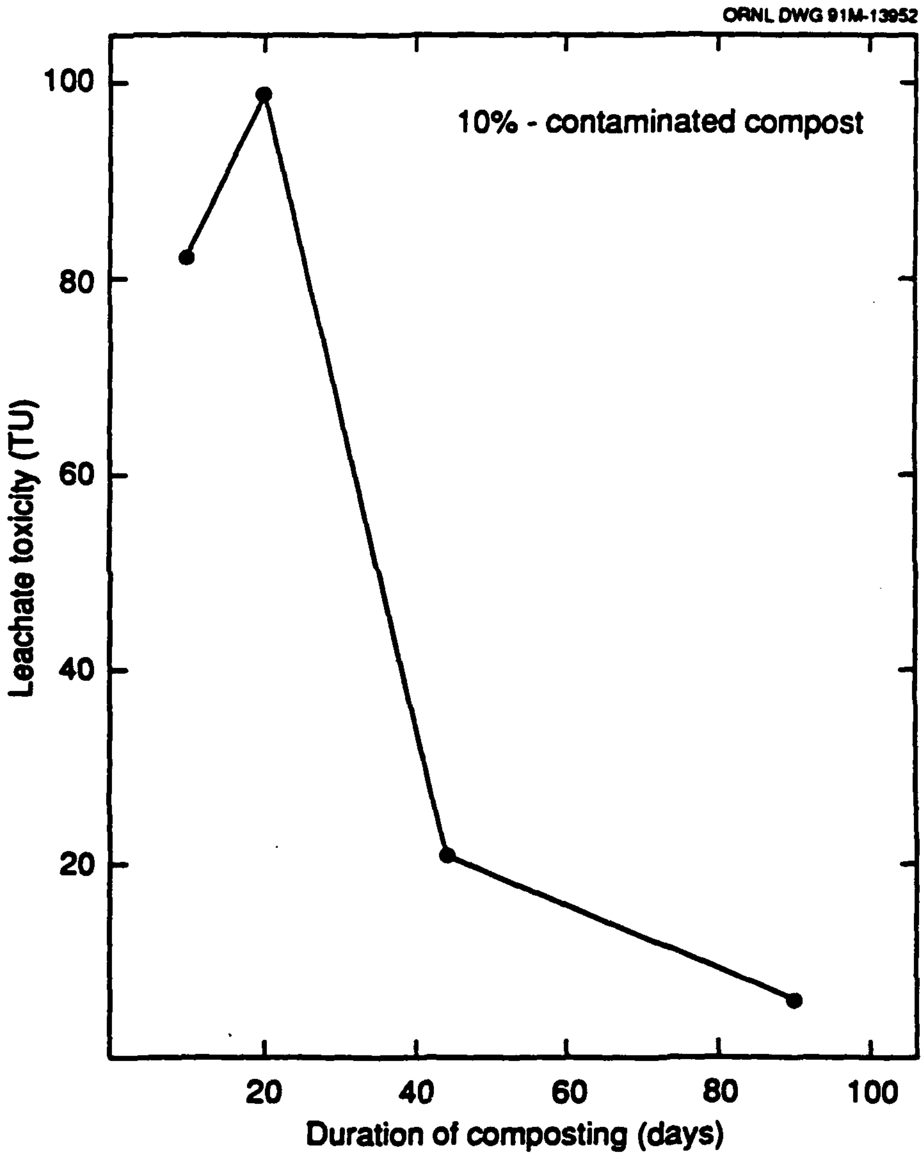
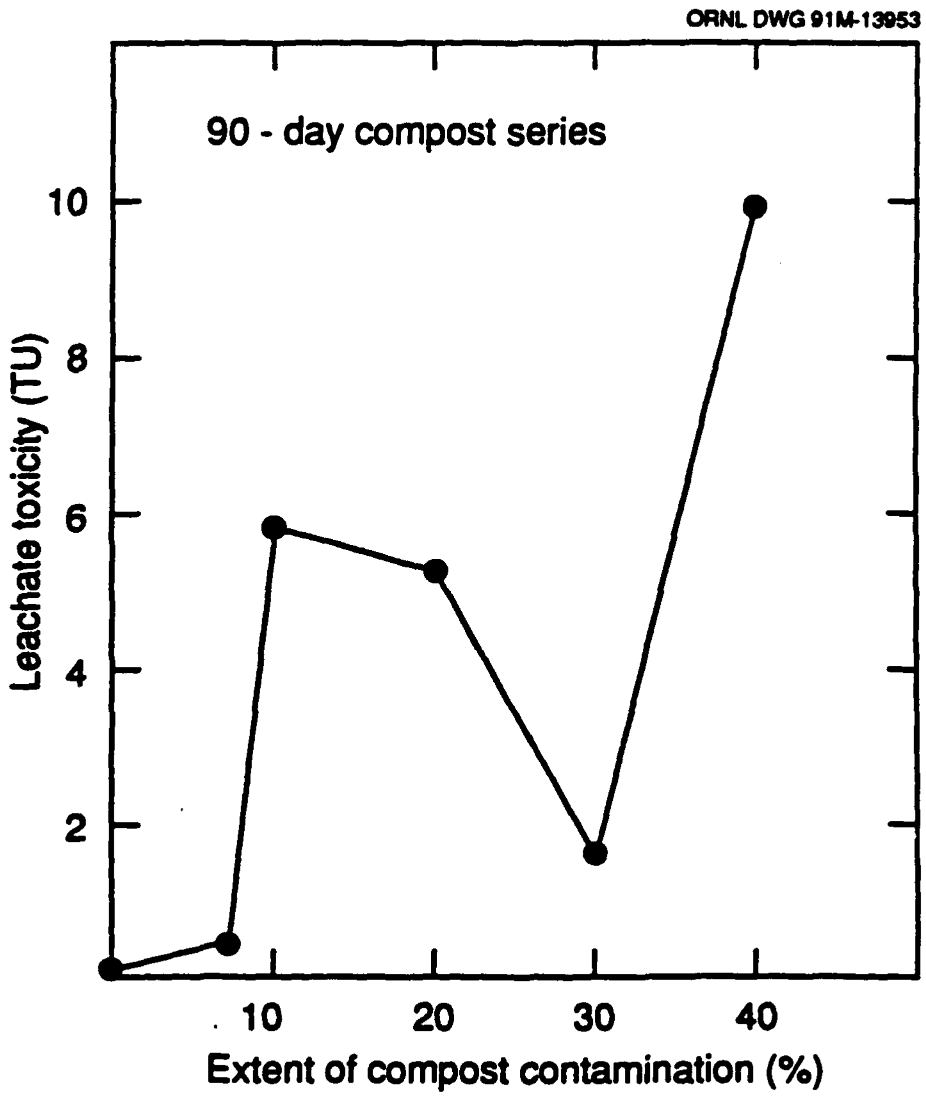


Fig. 3.2. Effect of initial concentration of TNT-contaminated soil (percentage, mass-to-mass basis) on toxicity of the leachate after composting for 90 d.



4 AMES MUTAGENICITY TESTING AND RAT ORAL TOXICITY SCREEN OF LEACHATES AND COMPOST EXTRACTS

As previously noted, the Ames test was developed as a bacterial screening assay for chemical mutagens. The assay detects back-mutation to histidine independence of mutant strains in the his operon of Salmonella typhimurium. Some strains of the bacteria can be reverted by base-pair substitutions (TA-100) or frameshift mutations (TA-98) and have been used to detect mutagens in a variety of complex mixtures. Results of Ames testing of aqueous leachates and organic solvent extracts of mesophilic and thermophilic composts from phase I of this study were previously reported (4).

The results indicated that composting was indeed an effective methodology for biotransformation of explosives in contaminated soil. Ames testing of both mesophilic and thermophilic compost piles indicated a marked reduction of mutagenic activity relative to the amount of activity expected from explosives concentrations in the original contaminated soil. Consequently a more detailed study including proper toxicological controls was undertaken at the Umatilla site. This study compared the efficacy of various amendment and soil mixtures and static pile versus mechanically mixed piles in the biotransformation of explosives.

4.1. Materials and Methods

Ames Bacterial Mutagenicity Test:

Preparation of histidine deficient agar plates, the addition of the Salmonella test strains, and the addition of compost leachates or extracts were carried out as described in the Phase I report (4). The Salmonella strains TA-98 and TA-100 used in the test have mutations in the rfa and uvrB genes. They also contain the R-factor plasmid pKM101. The genotypes of the tester strains were confirmed by evaluating their sensitivity to crystal violet and to UV light and resistance to ampicillin. Both strains were killed by exposure to crystal violet and UV irradiation but were unharmed by ampicillin, thus confirming their genotype.

The test strains were kept frozen in nutrient broth supplemented with 10% sterile glycerol at -80°C in 1 mL aliquots, each of which contained about 10^9 cells. For each experiment, 1 mL aliquots were inoculated into 30 mL of nutrient broth. The cultures were grown at 37°C unshaken for 6 hours, then gently shaken (120 rpm) for 10 hours. Histidine dependency was checked for each strain whenever experiments were performed.

In addition to their response to crystal violet, ampicillin, and UV irradiation, the Salmonella were also tested against known mutagens to confirm their sensitivity. The known mutagens, nitrofluorene, acetylaminoflurorene, benzo(a)pyrene, and sodium azide,

were tested with and without metabolic activation (rat liver microsomal fraction S-9). The effects of the known mutagens are shown in Table 4.1. The S-9 preparation was a rat liver S-9 with Aroclor activation, obtained from Litton Bionetics (Oklahoma City, OK). It was diluted 0.04 mL to 0.5 mL with salt solution before addition with the tester strains.

For statistical analysis, the dose/response data were analyzed by the SAS package to determine slopes over the linear portion of the data by the least squares method.

Rat Oral Toxicity Screen:

For testing of samples for overt toxicity we conducted a screen of the rat oral toxicity of the 100% contaminated soil (not composted, as a potential positive control), the 40% contaminated soil compost from day 90 (a "worst case" from the maximum soil % composted), the 10% uncontaminated soil compost from day 90 (to determine potential toxicity effects associated with the amendments), and the day 44 sample of the MC-3 mechanical pile compost. Nine week old male Sprague Dawley CD/CR rats (10 per group) were dosed once with 1 gram of sample by feeding the sample mixed in peanut butter. The rats were observed for mortality and signs of toxicity for two weeks. This was not a formal LD_{50} determination, but rather a relatively inexpensive screen to determine if oral toxicity was great enough to warrant a more extensive study.

4.2. Results and Discussion

Ames Bacterial Mutagenicity Test:

Problems arose in the initial tests of the CCLT leachates. Attempts to sterilize the samples by bath and probe ultrasonicators were only successful in sterilizing the 100% contaminated soil control, which was not composted with amendments. This suggested that the source of the bacterial contamination was the composting amendments. Autoclaving was considered, but ruled out since heating might either create or destroy mutagenic products in the leachate material.

Because there was no better alternative, filtration was tested as the method for sterilization of the CCLT leachates. Initially assayed were leachates from day 0 samples of 7, 10, 20, 30, and 40% soil composts, along with 10% uncontaminated soil compost and a 100% contaminated soil sample. No mutagenic activity was observed for any of the time 0 filtered samples (Table 4.2) except for the highest dose (160 µl) of 100% soil leachate. Fortunately, the 100% soil could be sterilized by sonication and thus filtered versus unfiltered could be compared. The 100% unfiltered had a slightly higher mutation rate than did the filtered, but both had low activity, detectable only at the highest dose. This comparison was beneficial because it demonstrated that the lack of mutagenicity in the leachates from the composts was most likely due to lower explosives content and not to

filtering, although filtering did remove some activity in the 100% soil sample leachate. Leachates from the 10% uncontaminated and 10% contaminated soil, and 100% soil samples were also tested after sterilization by filtration and yielded results (Table 4.3) similar to those seen at time 0. These initial results indicated the efficacy of filtration as a means of sterilizing the CCLT leachates. Subsequently all remaining CCLT leachates were similarly filtered and tested. As was previously noted in uncontaminated CCLT leachates from the LAAP site, little or no mutagenic activity was detected (Tables 4.4-4.5) even when mutagenicity was calculated from the highest dose applied to the plates. Most of the calculated activities were too low (or negative, because the number of revertants was less than the background) and cannot be considered significant.

In contrast to the CCLT leachates, the acetonitrile extracts of various compost samples yielded considerable mutagenic activity (Tables 4.6). Analysis of static pile samples showed a marked reduction in mutagenic activity over the ninety day composting period. The 7%, 10%, and 20% composted soil samples showed over a 90% reduction in mutagenic activity. Reduction of mutagenic activity in the 30 and 40 % soil piles was less dramatic. As was seen in the LAAP compost samples (4) the presence of the S9 activation system reduced the ability to detect mutagenic activity with the TA-98 and TA-100 Salmonella, and data presented here are only for experiments without S9. The full set of data are included in Appendix E. The mutagenic activity of most zero time static pile samples was more pronounced with the TA-100 test strain while the reverse was true with the 90 day samples.

The mechanically stirred compost piles proved more effective than static piles of comparable soil percentage in reducing mutagenic activity of the explosive contaminated soil. However, it could not be determined if this was due to the mechanical agitation per se since different amendments were used. More than 95% of the mutagenic activity was abolished in only 44 days in the MC-3 pile which contained 25% contaminated soil. Over 70% of the mutagenic activity with strain TA-98 was degraded in the MC-4 pile which contained 40% contaminated soil. As was seen in the static pile samples presence of the S9 activation system also interfered with detection of mutagenesis in the mechanical pile samples. Unlike the static pile samples the mechanically stirred pile samples were generally more reactive with the TA98 test strain.

Rat Oral Toxicity Screen:

No toxicity was observed in rats fed any of the various soil or composted soil samples. Since no toxicity was evident in noncomposted soil, amelioration of toxicity by composting could not be demonstrated.

Overall static pile composting of 10, 20 and to a degree 30% soil markedly reduced the mutagenic activity as did mechanical composting of 25% and to a degree 40% soil. Oral toxicity in rats was not apparent even in noncomposted soil.

4.3 Conclusions

1. As was observed in the Phase I study, CCLT leachates of explosives contaminated soil or composts showed little or no mutagenic activity.

2. Also, as seen previously, acetonitrile extracts of the contaminated soil and composts were mutagenic.

3. Composting of the contaminated soil at the UMDA site markedly reduced concentrations of mutagens especially in the 7, 10, and 20% composts and in the 25% soil mechanically stirred composts.

4. While the mechanically stirred composting appeared more effective than static composting in reducing mutagenicity, the difference might be attributed to the use of a different amendment.

5. No toxicity was detected in rats fed the explosives contaminated soil or composts.

Table 4.1. Results of Ames Tests of Known Mutagens

	TA-98, Rev./Plate		TA-100, Rev./Plate	
Sample	-S9	+S9	-S9	+S9
	-S9	+S9	-S9	+S9
CONTROL	25	NT	138	NT
Nitrofluorene[a]	291	NT	512	NT
Acetylaminofluorene[a]	NT	533	NT	227
Sodium Azide[b]	NT	NT	586	694
Benzo(a)pyrene[c]	NT	165	NT	694

NT = Not Tested
[a] = 10 μg/plate
[b] = 2 μg/plate
[c] = 5 μg/plate

Table 4.2. Results of Ames Test of Leachates of Day 0 Compost or Soil Samples

		Revertants/Plate			
		TA-98		TA-100	
Leachate or Sample	µL/plate	-S9	+S9	-S9	+S9
Spontaneous	-	23	NT	130	NT
B(a)P[a]	5	19	120	148	490
7% Soil[b]	10	24	28	143	152
	20	20	25	135	145
	40	21	24	134	147
	80	30	19	143	152
10% Soil[b]	10	30	25	149	171
	20	25	26	139	161
	40	27	25	142	152
	80	21	29	137	152
20% Soil[b]	10	22	24	156	158
	20	27	29	143	156
	40	27	23	144	145
	80	35	24	154	159

Table 4.2. Results of Ames Test of Leachates of Day 0 Compost or Soil Samples (Continued)

		Revertants/Plate			
		TA-98		TA-100	
Leachate or Sample	μL/plate	-S9	+S9	-S9	+S9
30% Soil[b]	20	40	23	138	124
	40	30	25	133	122
	80	35	27	147	140
	160	33	24	148	140
40% Soil[b]	20	37	36	208	219
	40	29	31	230	224
	80	30	31	232	226
	160	42	38	222	205
100% Soil[b]	20	29	36	228	208
	40	27	30	228	245
	80	48	33	265	229
	160	53	32	286	225
100% Soil[c]	20	51	NT	233	NT
	40	48	NT	224	NT
	80	50	NT	262	NT
	160	102	NT	386	NT

[a] = Known mutagen.
[b] = CCLT leachates filtered through 0.2 μm cellulose filter.
[c] = CCLT leachate sterilized by ultrasonication.

Table 4.3. Results of Ames Tests of Other CCLT Leachates

		Revertants/Plate			
		TA-98		TA-100	
Leachates[a] or Sample	μL/plate	-S9	+S9	-S9	+S9
Spontaneous	-	20	NT	123	NT
B(a)P	5	21	102	140	513
10%	20	29	NT	134	NT
Uncontaminated	40	24	NT	138	NT
Day 0	80	35	NT	140	NT
Filtered[b]	160	23	NT	109	NT
10% Soil	20	28	NT	146	NT
Day 10	40	34	NT	134	NT
	80	33	NT	139	NT
	160	36	NT	152	NT
100% Soil	20	23	NT	153	NT
	40	20	NT	151	NT
	80	36	NT	163	NT
	160	46	NT	198	NT

[a] Contaminated soil compost, all samples filtered through 0.2 μm cellulose filter.
[b] Unfiltered also tested, but plates were overgrown with bacterial contamination.

Table 4.4. Summary of Ames Tests of UMDA Static Pile Compost CCLT Leachates

		Revertants/mL of Leachate[a]			
		TA-98		TA-100	
Compost Leached	Composting Day	+S9	-S9	+S9	-S9
0	0	NT	47	NT	53
	90	-3	-6	50	81
7	0	13	22	69	41
	90	9	44	19	-13
10	0	19	6	69	22
	10	NT	41	NT	50
	90	19	34	0	44
20	0	3	41	91	75
	90	-3	50	69	69
30	0	22	47	53	75
	90	13	22	84	6
40	0	41	38	59	78
	90	31	28	200	253
100	-	47	94	69	181

[a] Data calculated from 80μL dose of leachate
NT = not tested

Table 4.5. Summary of Ames Test of UMDA Mechanical Composter CCLT Leachates

		Revertants/mL of Leachate[a]			
		TA-98		TA-100	
Compost Leached	Compost Day	+S9	-S9	+S9	-S9
MC-3 (25%)	0	38	50	63	144
	10	41	32	66	59
	20	6	3	3	-3
	44	19	19	34	3
MC-4 (40%)	0	13	9	78	13
	10	-9	22	47	9
	20	19	25	63	59
	44	22	16	75	56

[a] Data calculated for 80μL dose of Leachate.

Table 4.6. Specific Mutagenicity for UMDA Composts (Acetonitrile Extracts)

		Specific Mutagenicity, Rev/g	
		Avg ± Std. Dev.	
Compost	Days of Composting	TA-98 w/o S9	TA-100 w/o S9
Static Piles:			
0%	0	0	0
	10	37,500	18,800
	20	0	0
	44	0	0
	90	0	0
7%	0	83,200 ± 12,500	205,000 ± 5,780
	90	9,820 ± 610	2,100 ± 550
10%	0	87,200 ± 5,390	100,000 ± 2,750
	10	110,000 ± 9,200	56,300 ± 4,970
	20	97,500 ± 6,750	112,000 ± 4,920
	44	38,000 ± 5,400	27,400 ± 4,380
	90	14,300 ± 530	12,800 ± 1,140
20%	0	310,000 ± 30,700	546,000 ± 25,200
	90	21,600 ± 360	14,200 ± 1,100
30%	0	216,000 ± 16,100	350,000 ± 25,000
	90	51,900 ± 3,700	33,100 ± 1,030
40%	0	160,000± 9,490	286,000 ± 19,300
	90	86,900 ± 4,300	64,800 ± 2,030

Table 4.6. Specific Mutagenicity for UMDA Composts (Acetonitrile Extracts) (Continued)

		Specific Mutagenicity, Rev/g	
		Avg ± Std. Dev.	
Compost	Days of Composting	TA-98 w/o S9	TA-100 w/o S9
100% Soil (not composted)		284,000 ± 10,700	259,000 ± 30,900
Stirred Composters:			
ME-3 (25%)	0	344,000 ± 24,400	143,000 ± 13,200
	10	87,000 ± 14,500	44,200 ± 6,300
	20	18,100 ± 1,680	16,200 ± 4,860
	44	9,760 ± 660	3,200 ± 7,200
MC-4 (40%)	0	456,000 ± 21,200	170,000 ± 22,500
	10	77,500 ±7,470	89,400 ± 18,700
	20	67,700 ± 6,640	63,900 ± 7,660
	44	71,800 ± 4,570	52,600 ± 3,710

5 INTEGRATION OF RESULTS

5.1 Comparison of Chemical Analysis and Bacterial Mutagenicity

Both the analysis of explosives and TNT metabolites (Chapter 2) and the toxicological tests (Chapters 3 and 4) show the same trends in decontamination of soil by composting. The specific mutagenicity of the 10% soil compost and the concentrations of TNT and major metabolites are plotted as a function of composting time in Figure 5.1. For the first 20 days of composting, the mutagenicity as determined by both strains varied widely before dropping rapidly after 20 days. Simultaneously, the TNT dropped steadily and rapidly while the monoaminodinitrotoluene metabolites rose and then fell, and the diaminonitrotoluenes rose slowly. The TNT has much higher specific mutagenicity than any of the metabolites observed by HPLC, and it should be the controlling mutagen. However, no obvious one-to-one relationship between TNT concentration and mutagenicity was found.

A similar comparison of the mutagenicity of the final static pile composts (after 90 days of composting) and TNT/metabolites (Figure 5.2) also shows this qualitative relationship between chemistry and mutagenicity. As the volume percentage of contaminated soil in the compost was increased, the mutagenicity and the TNT/metabolites concentrations in the final composts increased. This was probably because of the increased dilution of the amendments by the increased volume percent of soil. The 100% soil (not composted - this was the starting material for composting) had both the greatest mutagenic activity and the highest concentration of TNT. No TNT metabolites were detected in the 100% soil.

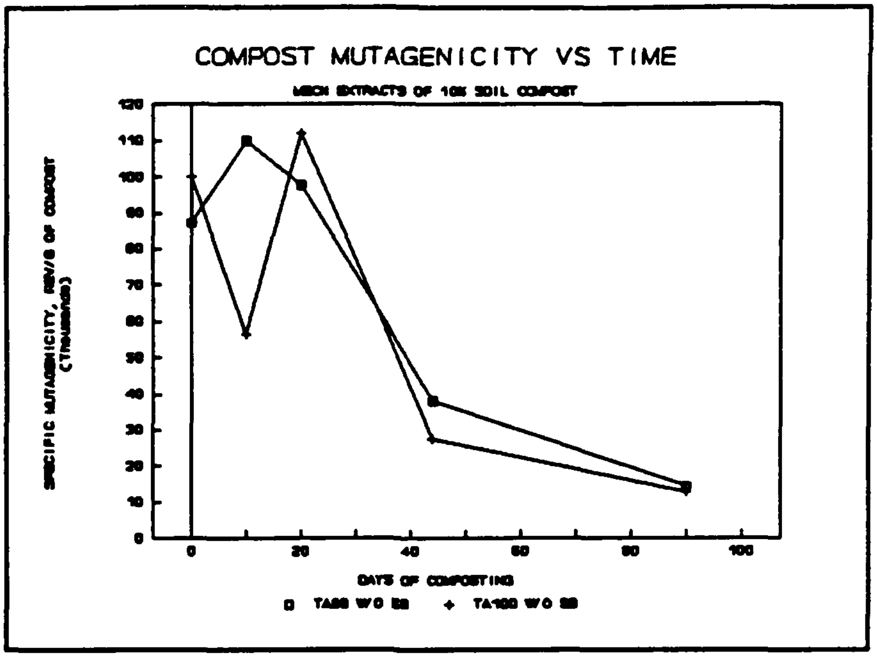
The measured mutagenicity was compared with the mutagenicity predicted from the concentrations of TNT and metabolites determined by HPLC. TNT is the most mutagenic of the compounds determined. The amino-metabolites of TNT are less active because the specific mutagenic activity decreases with increasing number of nitro groups reduced to amino groups. HMX and RDX do not have measurable bacterial mutagenicity (4) with these strains, and were not considered in this calculation. Table 5.1 lists the percentage of the mutagenic activity determined with strains TA-98 and TA-100 (without S9 metabolic activation) which was accounted for by TNT and its detectable metabolites. The accounted activity usually was a small fraction of the measured activity. The major observation is that with increasing biotransformation (through either longer composting time or a lower volume percentage of contaminated soil), a decreasing fraction of the mutagenic activity is accounted for. The control pile, composed from the same type of soil as the contaminated lagoon soil and from the same amendment mixture, did not exhibit detectable mutagenicity, and thus the amendments and soil do not appear to contribute to the mutagenicity. Therefore, the unaccounted mutagenicity must be due to either an undetected compound or compounds initially present in the contaminated soil and not biotransformed, or compounds created by biotransformation in the composting process. Synergism among mutagens and matrix effects also may affect the activity.

5.2 Comparison of Chemical Analysis and Toxicity to Ceriodaphnia dubia

Plots of the CCLT leachate toxicity and TNT/metabolites as a function of composting time for the 10% soil compost are compared in Figure 5.3. The same general trends as noted above for mutagenicity and chemistry are evident. The fecundity endpoint (plotted as the reciprocal of the EC_{50} to represent decreasing toxicity with a decreasing numerical value) varied (as did the mutagenicity of the compost) before dropping off steadily after 20 days of composting. This endpoint followed the general trend of the leachate TNT concentration. However, the survival endpoint (shown as the reciprocal of the LC_{50}) declined much more rapidly than either the fecundity or the TNT. The tests for the MC-3 and MC-4 compost leachates also showed this same behavior. For Ceriodaphnia and most other organisms, survival is a more fundamental necessity than fecundity: under increasing levels of stress, a healthy animal initially diverts metabolic energy away from reproduction and towards maintenance. Thus, the rapid decline of the survival endpoint (shown as the reciprocal of the LC_{50}), relative to that of fecundity, was to be expected.

In Figure 5.4, the toxicity (as $1/LC_{50}$ and $1/EC_{50}$) of the leachate from the final day 90 composts is compared with the leachate concentrations of TNT and its metabolites. In this figure, all of the $1/LC_{50}$s except for the 10% and 40% soil composts are maximum values because the LC_{50}s were determined as minimum values. As for compost mutagenicity, with increased volume percent of contaminated soil in the compost, the toxicity and TNT/metabolites concentrations of the final compost leachate increased. The leachate of the 100% contaminated soil was by far the most toxic, but it did not contain an appreciably higher TNT concentration than that of the 40% soil compost leachate (probably due to TNT aqueous solubility limitations). The former leachate did lack the TNT metabolites which were detected in the latter. This suggests that the metabolites in the 40% soil compost leachate did not increase the toxicity, and that other compounds must have controlled the toxicity.

Bacterial mutagenicity was not detected in the final compost of the control pile ("0%" contaminated soil, but actually 10% uncontaminated soil of the same type as the contaminated soil), but a low level of leachable toxicity to Ceriodaphnia was found. TNT and its metabolites were not detected in the leachate. This demonstrates that the soil/amendments mixture itself has some toxic properties. These could originate from the chicken manure (5) in the amendment mixture, and might be similar to animal feedlot runoff.



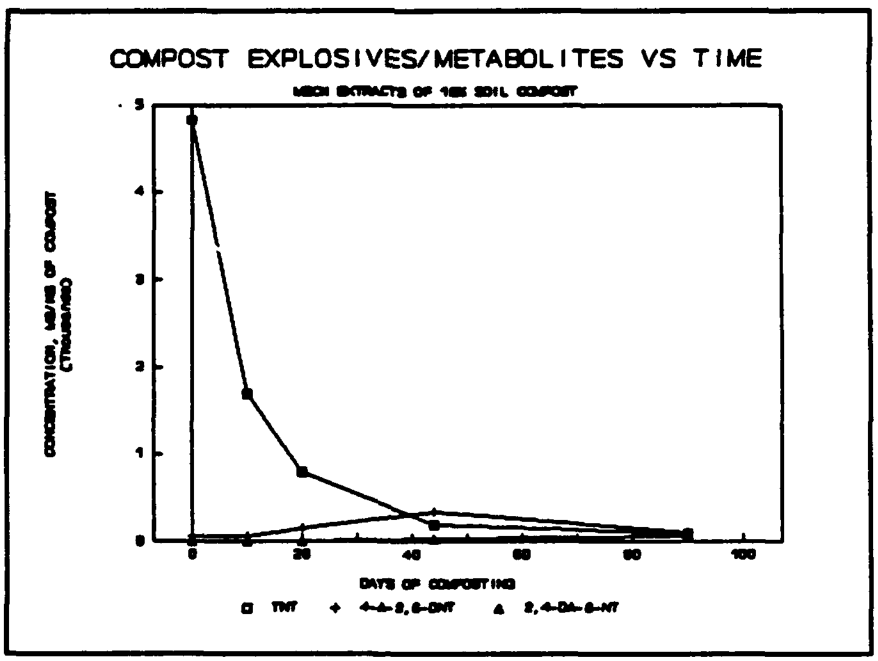
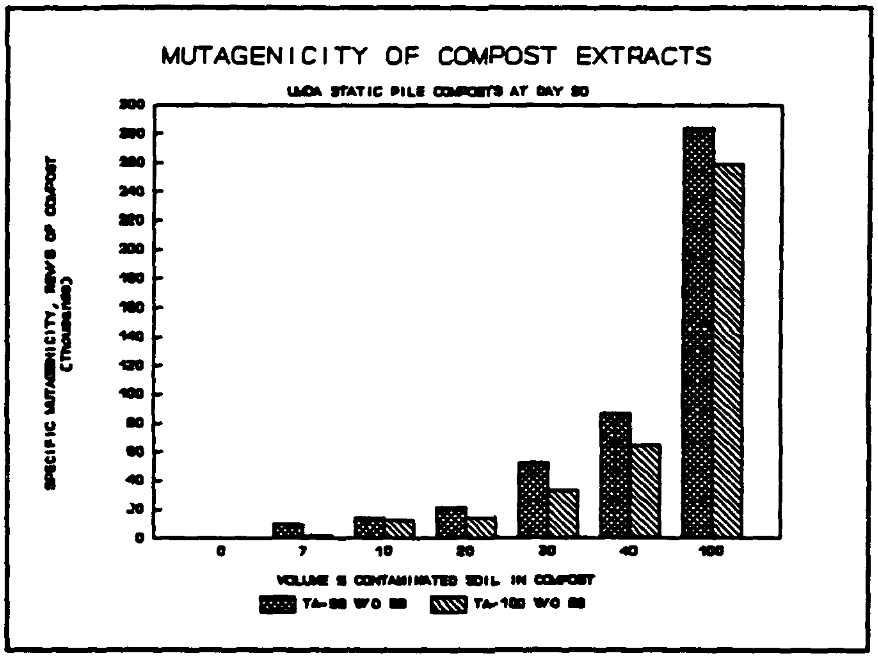


Figure 5.1 Comparison of 10% Soil Compost Mutagenicity and TNT/Metabolites Concentrations.



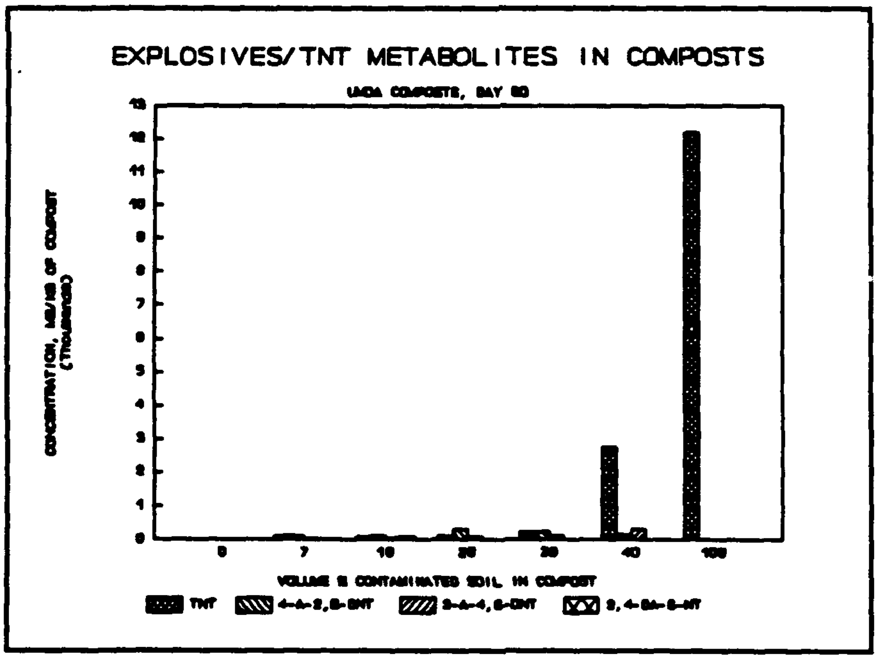
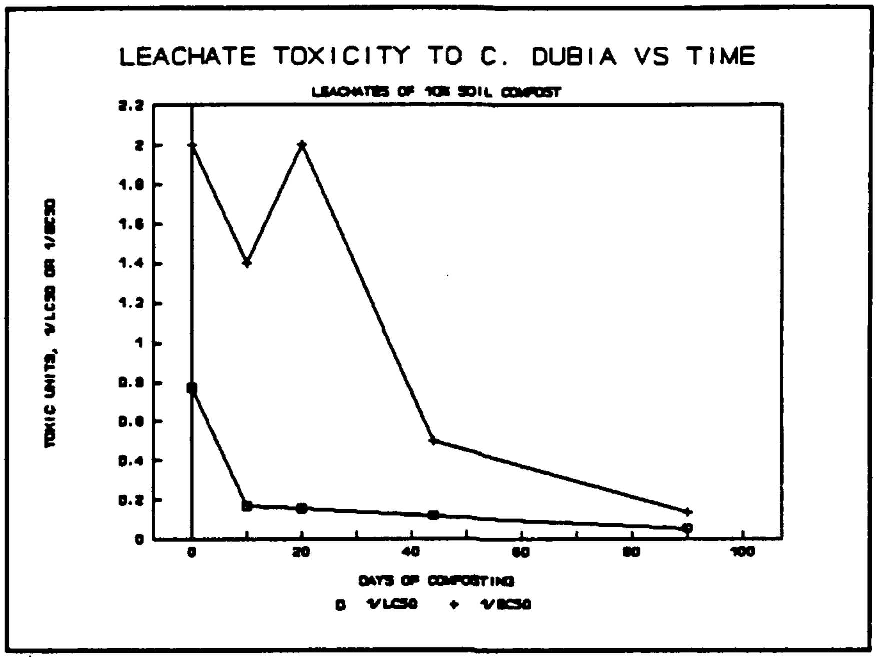


Figure 5.2 Comparison of Final Compost Mutagenicity and TNT/Metabolite Concentrations

Table 5.1 Accounting of Microbial Mutagenic Activity (Strain TA-98, TA-100 w/o S9) in Composts by TNT and Metabolites Determined by HPLC.

Compost	Mutagenicity Accounted for Strains TA-98, TA-100, %[a]				
	Day 0	Day 10	Day 20	Day 44	Day 90
7%	5, 3				5, 26
10%	18, 19	5, 12	3,3	3, 5	3, 4
20%	7, 5				3, 6
30%	14, 10				2, 4
40%	23, 16				14,23
100%	23, 31				
MC-3	<4, <13	<3, <10	<5, <10	<1,<3	
MC-4	6, 19	27, 29	12, 15	2, 3	

[a]Format is: % accounting of mutagenicity measured with strain TA-98 (w/o S9), % accounting of mutagenicity measured with strain TA-100 (w/o S9).



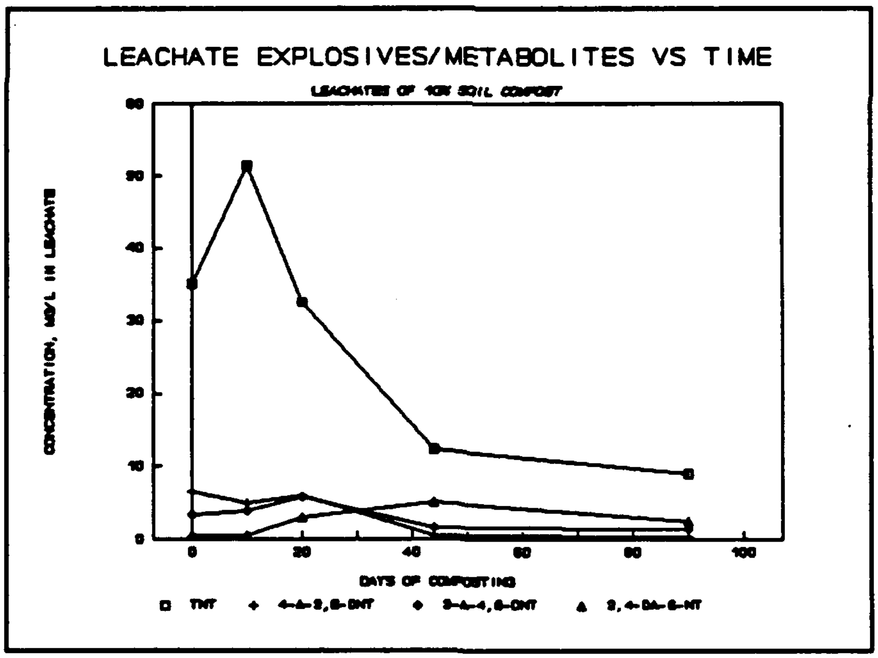
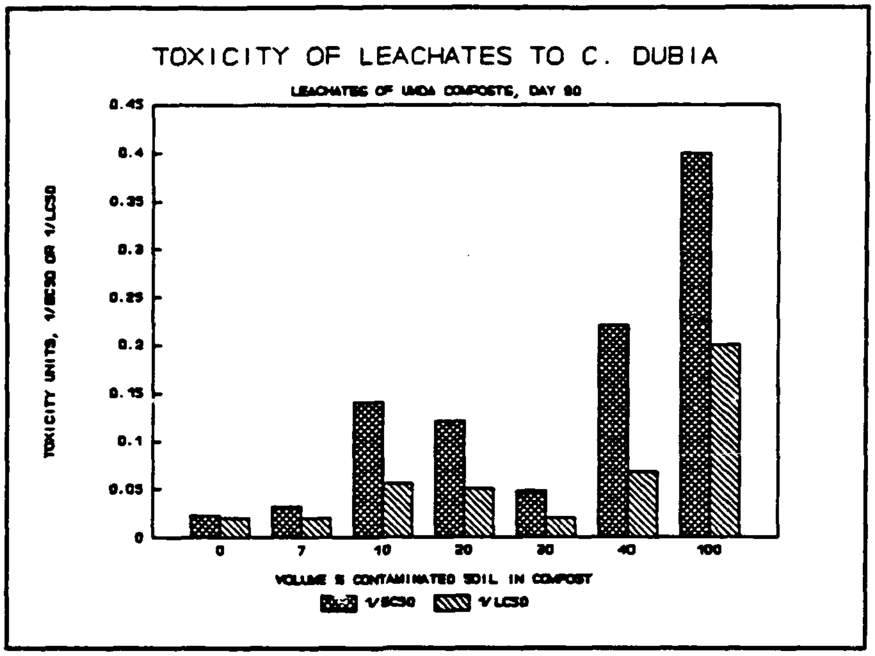


Figure 5.3 Comparison of 10% Soil Compost Leachate Toxicity and TNT/Metabolites Concentrations



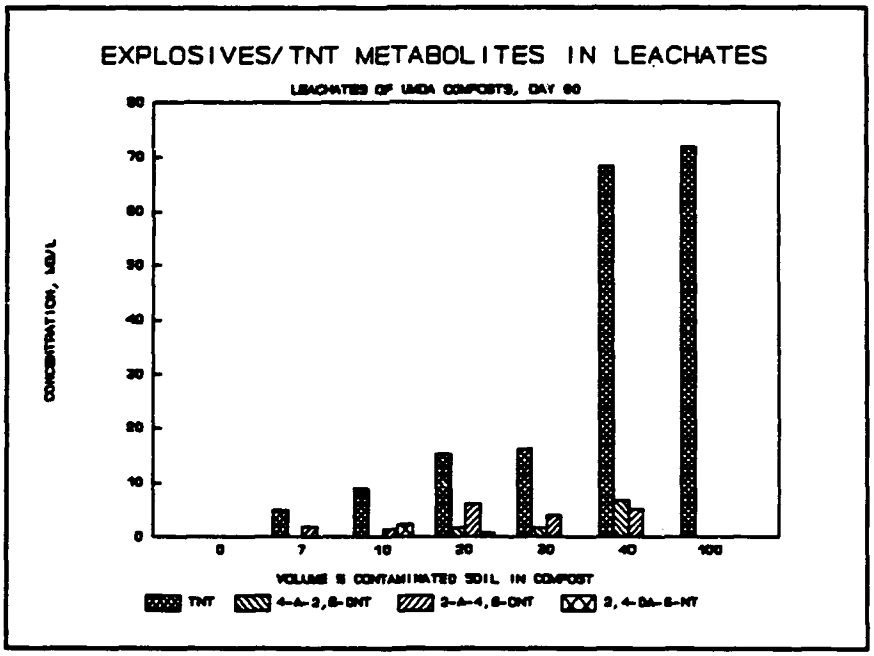


Figure 5.4 Comparison of Toxicity and TNT/Metabolites for Leachates of Final Composts. (Maximum values for $1/LC_{50}$s of all but 10% and 40% soil composts.)

5.3 Comparison of Composting Efficiency Measures

The efficiency of composting is summarized in Table 5.2. This table shows the percentage reduction in compost explosives, compost mutagenicity, and compost leachate toxicity achieved by replacing the "100% contaminated soil" removed from the dried lagoon with final compost product. Although this is a less scientific presentation than comparing the reduction in explosives and toxicity achieved by each compost pile, it does more realistically reflect the potential changes from site remediation by composting, i.e., from replacing contaminated soil with final compost. In Table 5.2, for a given column, the shaded area encloses the most efficient reductions, grouped together as being the same at the 5% significance level. The underlined data are the next most efficient, and again are grouped together at the 5% significance level.

It is apparent that TNT is relatively easy to transform, and all but the 40% soil static pile achieved a highly efficient reduction in TNT concentration. However, for HMX and RDX, the MC-3 (25% soil) mechanical stirred compost and the "new" 10% soil static pile were most efficient, followed by the 7% and 10% static pile composts. For HMX, the MC-3 and "new" 10% and 7% static piles were most efficient. The 7% static pile overlapped the next most efficient group, with the 10% and 20% static pile composts. For reduction of direct-acting bacterial mutagens, the MC-3 and 7% static pile were optimum for both tester strains. The "new" 10% static pile also probably would fit in this group, based upon its efficient reduction of explosives, but it was not tested. The 10% and the 10% and 20% static composts ranked next for the TA-98 and TA-100 strains, respectively. Resources were not large enough to replicate the Ceriodaphnia toxicity tests sufficiently to perform statistical tests on the data, but the professional judgement of the experienced toxicologist is that the break point in the composting (i.e., the point beyond which a significant drop occurred in composting efficiency) was $\geq$ 30 volume % soil in the static pile.

Overall, under the conditions used for the static piles, the 10% or 20% soil concentrations appear to be maximum; for the stirred composter, the 25% concentration was the better of the two. The much greater efficiency of the "new" 10% static pile versus the "old" 10% static pile suggests that even higher volume percentages of soil could be tolerated in the static piles if the second amendment were used.

Table 5.2 Comparison of the Percentage Decreases (Day 90 of Compost or Leachate) in Explosives, Bacterial Mutagenicity, and Toxicity to Ceriodaphnia dubia. (Shaded area encloses statistically similar data [for a given data column] at a 5% significance level. Next lower, similar data are underlined. For Ceriodaphnia toxicity, the toxicologist's judgement for equivalent data are shaded.)

Compost[a]	Explosives Conc.[b]			Mutagenicity[c]		Toxicity[d] to Ceriodaphnia dubia
	TNT	RDX	HMX	TA-98	TA-100	
40% MC	98.3	55.2	0	74.7	79.7	72
25% MC	99.9	97.2	75.0	96.6	98.8	88
10% NS[e]	99.7	96.7	85.0	ND	ND	ND
7% S	97.7	81.5	66.9	96.5	99.2	92
10% S	99.2	71.5	62.5	95.0	95.1	65
20% S	98.8	53.2	41.1	92.4	94.5	70
30% S	98.2	43.8	22.1	81.7	87.2	88
40% S	77.5	0	8.2	69.3	75.0	45
0% S[f]	NA	NA	NA	NA	NA	95

[a]Volume % contaminated soil in mechanical composter (MC) or static pile (S). NS refers to "new" static pile.
[b]Percent decrease in concentrations of explosives.
[c]Percent decrease in specific mutagenicity for tester strains TA-98 and TA-100 without S9 metabolic activation.
[d]Percent decrease in reproduction (as 1/EC50) of Ceriodaphnia dubia.
[e]Toxicity not determined.
[f]Explosives and mutagenicity not detected in control pile from uncontaminated soil.

5.4 Estimation of Compost and Leachate Toxicity to Humans

In the absence of human oral toxicity data for explosives, one approach for evaluating the potential for human health risk is the comparison of explosives in the leachates with values derived from their EPA Drinking Water Exposure Level (DWEL). The EPA DWELs are "a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur." (20). The DWELs are, TNT = 0.02 mg/L (20), RDX = 0.1 mg/L (21), and HMX = 2 mg/L (22). If it is assumed that the main route of exposure to the general public is from compost leachate contamination of drinking water, and that a 100-fold dilution of leachate in water supplies is a conservative dilution (note: RCRA sets 100-times the Drinking Water Standards as the Regulatory Limits) (23), then 100-fold the DWEL would appear to be a reasonable criteria for evaluation of the compost CCLT leachates.

Table 5.3. compares the concentration of TNT, RDX, and HMX in the compost CCLT leachates with 100-times their DWEL. Not all of the explosives could be measured in all of the leachates because of interferences or low concentrations, but the available data show HMX to be far below 100 X DWEL. The 2 mg/L for TNT is achieved only by the 25% soil mechanical composter, and possibly the 40% soil mechanical composter (< 3 mg/L). The new 10% soil static pile compost was not leached, but the compost data (Table 2.7) suggest that its leachate would pass this criterion. The same case appears to hold for RDX.

The overall conclusion here is that current composting technology can reduce soil explosives contamination to levels which are not likely to be of human concern from a standpoint of leachate toxicity.

Table 5.3. Comparison of 100 x DWEL and Concentrations of Explosives in CCLT Leachates of Composts

	mg/L		
100 x DWEL or Leachate	TNT	RDX	HMX
100 x DWEL	2.0	10	200
7% S	5.0	-	3.1
10% S	9.1	-	3.5
20% S	15.4	-	4.0
30% S	16.2	-	-
40% S	68.3	-	-
25% MC	<0.6	1.3	2.5
40% MC	<3.0	17.1	<14

5.5 Conclusions

The main conclusion from this study is that composting can effectively reduce the concentrations of explosives and bacterial mutagenicity in explosives-contaminated soil, and can reduce the aquatic toxicity of leachable compounds. Small levels of explosives and metabolites, bacterial mutagenicity, and leachable aquatic toxicity remain after composting. The ultimate fate of the biotransformed explosives [some of which may be bound to the compost], and the source(s) of residual toxicity and mutagenicity remain unknown.

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APPENDIX A
ABBREVIATIONS FOR EXPLOSIVES AND TNT METABOLITES

List of Abbreviations for Explosive Compounds and TNT Metabolites

Abbreviation	Full Name
2,6-DA-4-NT	2,6-Diamino-4-nitrotoluene
2,4-DA-6-NT	2,4-Diamino-6-nitrotoluene
2,4,6-TNBAlc	2,4,6-Trinitrobenzyl alcohol
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine or cyclotrimethylenetrinitramine
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine or cyclotetramethylenetetranitramine
1,3,5-TNB	1,3,5-Trinitrobenzene
1,3-DNB	1,3-Dinitrobenzene
2-A-4,6-DNT	2-Amino-4,6-dinitrotoluene
4-A-2,6-DNT	4-Amino-2,6-dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
2,4-DNT	2,4-Dinitrotoluene
TNT	1,3,5-Trinitrotoluene
Tetryl	N-methyl-N,2,4,6-Tetranitroaniline
4-OHA-2,6-DNT	4-Hydroxyamino-2,6-dinitrotoluene
Azoxydimer	2,2',6,6'-tetranitro-4,4'-azoxytoluene

APPENDIX B
EXPLOSIVES AND TNT METABOLITES IN INDIVIDUAL SAMPLES OF MC-4 AND NEW ST7 COMPOSTS

ANALYSIS OF TNT, HMX, AND RDX IN INDIVIDUAL
SAMPLES OF UMDA COMPOST
(Data for four samples listed when one sample
was received broken.)

			CONCENTRATION, $\mu g/g$		
COMPOST	COMPOSTING DAY		TNT	HMX	RDX
MC-4	0		6,740	438	693
			6,920	475	792
			6,920	470	777
			7,200	440	754
		Avg.	6,950	456	754
		Std. Dev.	190	19.5	43.6
		RSD, %	2.7	4.3	5.8
	10		3,880	594	928
			4,920	542	858
			5,380	492	817
			5,420	470	770
			5,880	515	844
		Avg.	5,100	522	843
		Std. Dev.	760	48.0	58.0
		RSD, %	15	9.2	6.9

ANALYSIS OF TNT, HMX, AND RDX IN INDIVIDUAL
SAMPLES OF UMDA COMPOST
(Four samples shown when one sample was received broken.)

			CONCENTRATION, $\mu g/g$		
COMPOST	COMPOSTING DAY		TNT	HMX	RDX
MC-4	20		1,563	622	855
			1,149	586	1,004
			2,365	652	641
			1,523	600	952
			2,324	677	748
		Avg.	1,785	627	840
		Std. Dev.	536	37.3	148
		RSD, %	30	5.9	18
	44		528	645	800
			118	579	544
			230	672	544
			89.7	474	544
			79.4	635	672
		Avg.	209	601	621
		Std. Dev.	188	78.7	114
		RSD, %	90	13	18

ANALYSIS OF TNT, HMX, AND RDX IN INDIVIDUAL
SAMPLES OF UMDA COMPOST
(Four samples shown when one sample was received broken.)

			CONCENTRATION, $\mu g/g$		
COMPOST	COMPOSTING DAY		TNT	HMX	RDX
ST-7	0		4,580	311	582
			3,480	288	595
			4,180	234	533
			3,140	396	762
		Avg.	3,850	307	618
		Std. Dev.	650	67.4	99.6
		RSD, %	17	22	16
	10		1,464	184	403
			1,648	233	490
			1,256	272	401
			480	192	406
			543	134	228
		Avg.	1,078	203	386
		Std. Dev.	536	52.2	95.8
		RSD, %	50	26	25

ANALYSIS OF TNT, HMX, AND RDX IN INDIVIDUAL
SAMPLES OF UMDA COMPOST
(Four samples shown when one sample was received broken.)

			CONCENTRATION, μg/g		
COMPOST	COMPOSTING DAY		TNT	HMX	RDX
ST-7	20		34	10.9	18.3
			75.8	100	118
			295	104	149
			120	94.9	133
			61.8	148	143
		Avg.	117	91.6	112
		Std. Dev.	104	49.8	53.8
		RSD, %	89	54	48
	44		87.7	42.8	29.2
			26.1	37.0	29.2
			8.1	31.1	17.6
			31.7	74.8	40.8
			42.2	89.9	97.9
		Avg.	39.2	55.1	42.9
		Std. Dev.	29.8	25.8	31.8
		RSD %	76	47	74

ANALYSIS OF TNT, HMX, AND RDX IN INDIVIDUAL
SAMPLES OF UMDA COMPOST
(Four samples shown when one sample was received broken.)

			CONCENTRATION, µg/g		
COMPOST	COMPOSTING DAY		TNT	HMX	RDX
ST-7	90		30.3	63.8	40.5
			94.9	95.8	65.1
			15.7	24.4	24.3
			33.8	51.2	46.8
			29.0	70.6	54.6
		Avg.	40.7	61.2	46.3
		Std. Dev.	31.0	26.2	15.3
		RSD, %	76	43	33

APPENDIX C
STATISTICAL ANALYSIS
OF EXPLOSIVES DATA

Table C-1. Explosive concentrations in UMDA composts: μg/g of compost.

Obs	Explosive	% Soil	Day	Rep 1	Rep 2	Rep 3	Avg	St. Dev.	Variance	Detection Limit
1	HMX	7	0	243.7	205.5	209.9	219.7	20.9	436.8	133.5
2	HMX[a]	10	0	203.1	203.1	203.1	203.1	83.2	6915.6	267.0
3	HMX	20	0	291.2	349.4	319.3	320.0	29.1	847.1	267.0
4	HMX	30	0	296.0	314.9	275.8	295.6	19.6	382.3	267.0
5	HMX	40	0	313.4	352.1	355.7	340.4	23.5	550.0	267.0
6	HMX[a]	100	0	409.2	409.2	409.2	409.2	39.1	1526.4	445.0
7	HMX	7	90	167.8	116.4	122.6	135.6	28.1	787.2	44.5
8	HMX	10	90	159.7	144.9	155.3	153.3	7.6	57.8	44.5
9	HMX	20	90	242.3	242.1	239.0	241.1	1.9	3.4	66.8
10	HMX	30	90	304.6	317.6	334.5	318.9	15.0	224.8	66.8
11	HMX	40	90	376.6	370.9	379.0	375.5	4.2	17.3	178.0
12	RDX	7	0	717.3	792.3	775.8	761.8	39.4	1553.2	337.0
13	RDX	10	0	860.2	953.8	913.2	909.1	46.9	2203.1	67.4
14	RDX	20	0	998.3	1177.8	1136.4	1104.2	94.0	8834.3	67.4
15	RDX	30	0	1010.3	1090.3	992.2	1030.9	52.2	2725.2	67.4
16	RDX	40	0	1188.4	1231.1	1313.6	1244.4	63.6	4050.8	67.4
17	RDX	100	0	1248.5	1556.0	1348.5	1384.3	156.9	24602.1	112.3
18	RDX	7	90	317.4	214.3	235.0	255.6	54.5	2974.6	33.7
19	RDX	10	90	405.8	397.0	382.4	395.1	11.8	139.7	33.7
20	RDX	20	90	649.4	633.1	659.8	647.4	13.5	181 1	96.3
21	RDX	30	90	721.2	785.4	828.0	778.2	53.8	2890.4	134.8
22	RDX	40	90	1269.9	1520.8	1526.5	1439.1	146.5	21471.1	674.0
23	TNT	7	0	1134.4	1441.6	1138.1	1238.0	176.3	31083.0	104.0
24	TNT	10	0	4278.5	5443.0	4756.5	4826.0	585.4	342637.7	520.0
25	TNT	20	0	6064.2	6933.4	6657.8	6551.8	444.2	197304.2	520.0
26	TNT	30	0	8185.9	7966.8	7700.0	7950.9	243.3	59214.3	520.0
27	TNT	40	0	8546.7	9391.9	10291.2	9409.9	872.4	761064.0	520.0
28	TNT	100	0	10354.0	13743.9	12465.2	12187.7	1711.9	2930610.2	693.3
29	TNT	7	90	629.8	104.9	102.7	279.1	303.7	92226.5	10.4
30	TNT	10	90	158.4	61.1	70.3	96.6	53.7	2885.6	10.4
31	TNT	20	90	166.8	121.7	141.0	143.2	22.6	512.0	29.7
32	TNT	30	90	233.3	176.8	254.5	221.5	40.2	1613.2	41.6
33	TNT	40	90	2562.9	2793.3	2884.5	2746.9	165.7	27471.4	208.0

a) Values reported as below the detection level but average and standard deviation were also reported.

Table C-2. Averagae and Standard Deviations of Explosive Concentrations in UMDA Composts: $\mu g/g$ of Compost.

Percent Soil	Explosive ($\mu g/g$ of compoyst)					
	HMX		RDX		TNT	
	Day		Day		Day	
	0	90	0	90	0	90
7%	219.7	135.6	761.8	255.6	1238.0	279.1
	20.9	28.1	39.4	54.5	176.3	303.7
10%	203.1	153.3	909.1	395.1	4826.0	96.6
	83.2	7.6	46.9	11.8	585.4	53.7
20%	320.0	241.1	1104.2	647.4	6551.8	143.2
	29.1	1.9	94.0	13.5	444.2	22.6
30%	295.6	318.9	1030.9	778.2	7950.9	221.5
	19.6	15.0	52.2	53.8	243.3	40.2
40%	340.4	375.5	1244.4	1439.1	9409.9	2746.9
	23.5	4.2	63.6	146.5	872.4	165.7
100%	409.2	-	1384.3	-	12187.7	-
	39.1		156.9	-	1711.9	-

Table C-3. Lower 95% confidence interval, percent decrease from 100% soil, and upper 95% confidence interval for explosive data in UMDA composts.

OBS	EXPLOSIVE	DAY	7% Soil	10% Soil	20% Soil	30% Soil	40%Soil
1	HMX	0	33.15	10.77	4.71	12.16	-0.37
1	HMX	0	46.31	50.37	21.80	27.76	16.81
1	HMX	0	59.47	89.96	38.89	43.36	34.00
2	HMX	90	54.24	53.16	27.11	5.29	-13.23
2	HMX	90	66.86	62.54	41.08	22.07	8.24
2	HMX	90	79.49	71.91	55.05	38.84	29.70
3	RDX	0	29.63	16.68	0.40	6.09	-12.11
3	RDX	0	44.97	34.33	20.23	25.53	10.11
3	RDX	0	60.30	51.98	40.07	44.96	32.32
4	RDX	90	72.27	63.24	40.03	28.22	-29.39
4	RDX	90	81.54	71.46	53.23	43.78	-3.96
4	RDX	90	90.80	79.68	66.44	59.35	21.48
5	TNT	0	84.89	45.06	27.60	12.31	-1.21
5	TNT	0	89.84	60.40	46.24	34.76	22.79
5	TNT	0	94.79	75.74	64.88	57.21	46.79
6	TNT	90	91.81	98.08	98.21	97.15	69.05
6	TNT	90	97.71	99.21	98.83	98.18	77.46
6	TNT	90	103.61	100.33	99.44	99.22	85.87

t-Statistic for the difference between composts and 100% soil

OBS	ANALYTE	DAY	TSTAT07	TSTAT10	TSTAT20	TSTAT30	TSTAT40
1	HMX	0	7.40	3.88	3.17	4.50	2.61
2	HMX	90	9.84	11.13	7.44	3.73	1.48
3	RDX	0	6.66	5.03	2.65	3.70	1.43
4	RDX	90	11.77	10.89	8.10	6.33	-0.44
5	TNT	0	11.02	7.05	5.52	4.24	2.50
6	TNT	90	11.86	12.23	12.19	12.10	9.51

One-sided 5% significant t-Value for unequal variance

Table D-3. (continued)

OBS	ANALYTE	DAY	TVAL07	TVAL10	TVAL20	TVAL30	TVAL40
1	HMX	0	2.34	2.41	2.18	2.37	2.27
2	HMX	90	2.20	2.78	2.91	2.52	2.88
3	RDX	0	2.71	2.64	2.28	2.59	2.49
4	RDX	90	2.57	2.90	2.89	2.58	2.13
5	TNT	0	2.88	2.58	2.70	2.84	2.36
6	TNT	90	2.81	2.92	2.92	2.92	2.88

One-sided 1% significant t-Value for unequal variance

OBS	ANALYTE	DAY	TTVAL07	TTVAL10	TTVAL20	TTVAL30	TTVAL40
1	HMX	0	4.47	4.75	3.92	4.61	4.25
2	HMX	90	3.97	6.34	6.92	5.20	6.75
3	RDX	0	6.00	5.70	4.25	5.50	5.07
4	RDX	90	5.41	6.86	6.83	5.43	3.76
5	TNT	0	6.77	5.44	5.95	6.61	4.57
6	TNT	90	6.43	6.95	6.96	6.95	6.79

OBS	ANALYTE	DAY	DF07	DF10	DF20	DF30	DF40
1	HMX	0	3.06	2.84	3.70	2.95	3.28
2	HMX	90	3.63	2.15	2.01	2.58	2.05
3	RDX	0	2.25	2.35	3.27	2.44	2.64
4	RDX	90	2.48	2.02	2.03	2.46	3.98
5	TNT	0	2.04	2.46	2.27	2.08	2.97
6	TNT	90	2.13	2.00	2.00	2.00	2.04

For equal variance: t(0.95,4) = 2.1318, t(0.975,4) = 2.7764, t(0.99,4) = 3.7469

Table C-4. Explosive concentrations in UMDA composts: µg/g of compost.

Obs	Compost	Explosive	Day	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	N	Avg	St. Dev.
1	MC3	HMX	44	96.0	111.2	100.0	.	.	3	102.4	79
2	MC3	RDX	44	37.2	43.2	36.0	.	.	3	38.8	39
3	MC3	TNT	44	8.0	8.0	8.0	.	.	3	8.0	00
4	MC4	HMX	44	645.1	578.8	671.2	473.8	635.0	5	600.8	786
5	MC4	RDX	44	800.0	544.0	544.0	544.0	672.0	5	620.8	1145
6	MC4	TNT	44	528.0	117.7	229.7	89.7	79.4	5	208.9	1881
7	ST7	HMX	90	63.8	95.8	24.4	51.2	70.6	5	61.2	262
8	ST7	RDX	90	40.5	65.1	24.3	46.8	54.6	5	46.3	153
9	ST7	TNT	90	30.3	94.9	15.7	33.8	29.0	5	40.7	310

Table C-5. Average and standard deviations of explosive concentrations in UMDA composts: µg/g of compost.

	Explosive (µg/g of compost)					
	HMX		RDX		TNT	
	Day		Day		Day	
Compost	44	90	44	90	44	90
MC-3	102.4	.	38.8	.	8.0	.
	7.9	.	3.9	.	0.0	.
MC-4	600.8	.	620.8	.	208.9	.
	78.6	.	114.5	.	188.1	.
ST-7	.	61.2	.	46.3	.	40.7
	.	26.2	.	15.3	.	31.0

Table C-6. Lower 95% confidence interval, percent decrease from 100% soil, and upper 95% confidence interval for explosive data in UMDA composts.

OBS	Samp	Analyte	Soil	% Day	95% Confidence Limits Lower	% Diff	Upper	t-test	One-sided Percentiles 5%	1%	DF
1	MC3	HMX	25	44	67.87	74.98	82.08	13.32	2.78	6.29	2.16
2	MC3	RDX	25	44	96.14	97.20	98.25	14.85	2.92	6.95	2.00
3	MC3	TNT	25	44	99.91	99.93	99.96	12.32	2.92	6.96	2.00
4	MC4	HMX	40	44	-75.77	-46.82	-17.88	-4.59	1.95	3.15	5.95
5	MC4	RDX	40	44	40.90	55.15	69.41	7.34	2.27	4.21	3.31
6	MC4	TNT	40	44	95.30	98.29	101.27	12.08	2.89	6.83	2.03
7	ST7	HMX	10	90	75.75	85.04	94.34	13.68	2.32	4.41	3.11
8	ST7	RDX	10	90	94.35	96.66	98.96	14.73	2.90	6.86	2.02
9	ST7	TNT	10	90	99.16	99.67	100.17	12.29	2.92	6.96	2.00

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APPENDIX D
CERIODAPHNIA TOXICITY DATA

APPENDIX D. SUMMARY OF RESULTS OF <u>Ceriodaphnia</u> TOXICITY TESTS OF COMPOST LEACHATES.

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
Oct 18	Control	100	100	25.0 ± 4.5
"	CCLT blank	90	100	22.3 ± 2.7
"	"	70	90	22.3 ± 2.2
"	"	50	100	22.3 ± 3.8
"	"	30	90	23.0 ± 2.1
"	"	10	100	22.0 ± 4.4
			100	21.5 ± 3.9
"	7% cont., 0 d	90	0	— ± —
"	"	70	0	— ± —
"	"	50	0	— ± —
"	"	30	0	— ± —
"	"	10	50	0.0 ± —
"	"	5	100	0.2 ± 0.4
Nov 1	Control	100	100	30.8 ± 9.1
"	10% noncon., 0 d	20	100	12.6 ± 2.9
"	"	10	100	24.3 ± 2.5
"	"	5	100	29.8 ± 4.0
"	"	2.5	100	32.2 ± 4.2
"	"	1	90	30.2 ± 3.8
"	40% cont., 0 d	20	0	— ± —
"	"	10	0	— ± —
"	"	5	0	— ± —
"	"	2.5	100	3.8 ± 1.9
"	"	1	100	17.9 ± 3.8
"	"	0.5	0	— ± —

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	30% cont., 0 d	20	0	— ± —
"		10	0	— ± —
"		5	10	0.0 ± —
"		2.5	100	0.0 ± —
"		1	100	16.0 ± 4.9
"		0.5	0	— ± —
"	20% cont., 0 d	20	0	— ± —
"	"	10	0	— ± —
"	"	5	20	0.0 ± —
"	"	2.5	90	0.0 ± —
"	"	1	100	13.1 ± 4.4
"	"	0.5	20	12.0 ± 16.9
"	MC-10% cont., 0 d	20	0	— ± —
"	"	10	0	— ± —
"	"	5	50	0.0 ± —
"	"	2.5	90	0.0 ± —
"	"	1	100	10.5 ± 7.0
	"	0.5	100	22.8 ± 4.9
Nov 14	Control	100	90	28.9 ± 3.3
"	Noncon., 10 d	20	30	1.3 ± 1.5
"	"	10	90	9.3 ± 3.3
"	"	5	90	15.9 ± 3.6
"	"	2.5	100	27.5 ± 4.8
"	"	1	100	39.4 ± 7.5
"	"	0.5	100	39.5 ± 9.2
"	10% cont., 10 d	20	0	— ± —
"	"	10	0	— ± —
"	"	5	80	1.0 ± 1.9

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	"	2.5	90	3.4 ± 1.3
"	"	1	90	13.9 ± 7.5
"	"	0.5	100	36.0 ± 7.2
"	100% cont.	5	100	0.8 ± 1.6
"		1	100	22.9 ± 2.6
"		0.5	100	24.1 ± 2.4
"		0.1	100	24.2 ± 6.3
"		0.05	100	21.1 ± 9.8
Dec 6	Control	100	90	29.9 ± 10.2
"	10% cont.	20	0	— ± —
"		10	0	— ± —
"		5	0	— ± —
"		2.5	0	— ± —
"		1	70	0.0 ± 0
"		0.5	100	10.9 ± 3.0
Feb 28	20% Cont., 90 d	0.5	100	32.5 ± 5.4
"	"	1.0	100	31.8 ± 8.8
"	"	2.5	100	33.1 ± 7.9
"	"	5.0	90	28.6 ± 6.7
"	"	10.0	90	6.8 ± 1.9
"	"	20.0	90	0.4 ± 0.9
"	Control	—	80	27.3 ± 4.8
Mar 7	10% Cont., 90 d	0.5	100	37.0 ± 9.2
"	"	1.0	100	34.5 ± 8.2
"	"	2.5	100	32.8 ± 10.8
"	"	5.0	100	21.1 ± 5.8
"	"	10.0	100	7.0 ± 2.5
"	"	20.0	40	3.0 ± 2.9

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	Control	—	100	29.9 ± 8.1
Mar 14	10% Cont., 20 d	0.5	100	9.2 ± 6.6
"	"	1.0	90	2.8 ± 0.9
"	"	2.5	90	0.2 ± 0.4
"	"	5.0	70	0
"	"	10.0	0	-
"	"	20.0	0	0
"	Control	—	100	23.8 ± 3.3
Mar 14	Noncon., 20 d	0.5	90	19.9 ± 9.9
8"	"	1.0	80	19.9 ± 8.9
"	"	2.5	100	13.8 ± 6.4
"	"	5.0	100	2.4 ± 2.1
"	"	10.0	60	0.5 ± 1.2
"	"	20.0	70	0
"	Control	—	100	23.8 ± 3.3
Mar 20	10% Cont., 44 d	0.5	90	26.4 ± 12.6
"	"	1.0	80	33.0 ± 8.6
"	"	2.5	80	12.3 ± 7.3
"	"	5.0	70	8.9 ± 5.4
"	"	10.0	40	4.8 ± 5.1
"	"	20.0	10	0
"	Control	—	100	38.6 ± 4.0
Apr 4	10% cont., 10 d	0.5	90	18.9 ± 4.1
"	"	1.0	100	4.2 ± 1.9
"	"	2.5	90	1.2 ± 1.6
"	"	5.0	60	0
"	"	10.0	0	0
"	"	20.0	0	0

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	Control	—	100	24.3 ± 3.0
Apr 11	30% Cont., 90 d	0.5	90	34.8 ± 3.2
"	"	1.0	100	35.1 ± 5.3
"	"	2.5	100	37.2 ± 4.1
"	"	5.0	100	36.7 ± 4.6
"	"	10.0	100	36.8 ± 6.8
"	"	20.0	100	24.9 ± 6.0
Apr 11	40% Cont., 90 d	0.5	100	28.0 ± 8.9
"	"	1.0	100	25.0 ± 6.2
"	"	2.5	100	24.3 ± 7.6
"	"	5.0	100	13.4 ± 2.9
"	"	10.0	100	0.0 ± —
"	"	20.0	0	— ± —
"	Control	—	90	30.1 ± 7.8
May 2	Noncon., 90 d	10.0	100	34.8 ± 11.8
"	"	20.0	100	35.6 ± 4.9
"	"	30.0	90	24.5 ± 10.7
"	"	40.0	90	21.8 ± 12.2
"	"	50.0	100	17.6 ± 12.2
"	Control	—	100	41.0 ± 5.7
May 9	7% Cont., 90 d	10.0	100	28.2 ± 6.7
"	"	20.0	100	27.0 ± 4.0
"	"	30.0	100	19.5 ± 6.6
"	"	40.0	100	9.6 ± 6.1
"	"	50.0	100	9.3 ± 5.8
"	Control	—	90	36.0 ± 8.2
May 30	30% Cont., 90 d	10.0	100	23.0 ± 4.2
"	"	20.0	90	14.6 ± 9.9

Table 1. (continued)

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	"	30.0	100	0.6 ± 1.1
"	"	40.0	100	0.0 ± —
"	"	50.0	70	0.0 ± —
"	Control	—	100	26.5 ± 3.5
June 6	Noncont., 44 d	10.0	100	36.3 ± 9.5
"	"	20.0	100	39.7 ± 4.4
"	"	30.0	100	36.3 ± 3.9
"	"	40.0	90	31.8 ± 9.7
"	"	50.0	100	28.0 ± 8.4
"	Control	—	100	34.6 ± 6.6
June 13	40% MC-4, 0 d	0.5	100	33.4 ± 4.9
"	"	1.0	100	25.9 ± 6.3
"	"	2.5	100	1.2 ± 0.4
"	"	5.0	0	— ± —
"	"	10.0	0	— ± —
"	"	20.0	0	— ± —
"	Control	—	90	44.6 ± 2.7
June 13	40% MC-4, 10 d	0.5	100	35.1 ± 5.1
"	"	1.0	90	29.8 ± 4.1
"	"	2.5	100	4.7 ± 3.5
"	"	5.0	0	— ± —
"	"	10.0	0	— ± —
"	"	20.0	0	— ± —
"	Control	—	90	44.6 ± 2.7
July 11	40% MC-4, 20 d	0.5	70	3.1 ± 1.6
"	"	1.0	80	4.8 ± 5.9
"	"	2.5	90	9.0 ± 4.0

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	"	5.0	100	10.3 ± 3.9
"	"	10.0	0	— ± —
"	"	20.0	0	— ± —
"	Control	—	100	19.1 ±
Jul 11	40% MC-4, 44 d	0.5	100	8.6 ± 8.1
"	"	1.0	90	6.9 ± 6.2
"	"	2.5	90	6.3 ± 5.4
"	"	5.0	80	3.9 ± 4.0
"	"	10.0	80	5.4 ± 4.8
"	"	20.0	100	0.2 ± 0.6
"	Control	—	100	19.1 ± 6.0
Aug 1	30% MC-3, 0 d	0.5	80	4.0 ± 1.2
"	"	1.0	60	3.5 ± 2.3
"	"	2.5	90	0.0 ± —
"	"	5.0	20	0.0 ± —
"	"	10.0	0	—
"	"	20.0	0	—
"	Control	—	100	24.9 ± 5.7
Aug 1	30% MC-3, 10 d	0.5	100	28.5 ± 1.5
"	"	1.0	100	24.8 ± 2.6
"	"	2.5	90	12.7 ± 6.9
"	"	5.0	70	1.4 ± 1.3
"	"	10.0	60	1.5 ± 2.0
"	"	20.0	0	—
"	Control	—	100	24.9 ± 5.7
Aug 18	30% MC-3, 20 d	0.5	90	25.2 ± 3.4
"	"	1.0	100	24.4 ± 7.5
"	"	2.5	100	18.4 ± 7.0

Test date	Sample	Leachate conc. (%)	Survival (%)	Repro. (mean ± SD)
"	"	5.0	90	17.7 ± 4.4
"	"	10.0	100	6.8 ± 3.9
"	"	20.0	90	3.3 ± 1.6
"	Control	—	80	28.6 ± 2.6
Aug 18	30% MC-3, 44 d	0.5	100	26.0 ± 4.6
"	"	1.0	90	24.2 ± 6.9
"	"	2.5	100	20.0 ± 6.2
"	"	5.0	90	20.0 ± 5.4
"	"	10.0	100	18.0 ± 3.6
"	"	20.0	100	14.4 ± 5.3
"	Control	—	80	28.6 ± 2.6
Sept 13	40% MC-4", 44 d	0.5	100	23.6 ± 5.4
"	"	1.0	90	28.7 ± 6.8
"	"	2.5	90	24.5 ± 5.9
"	"	5.0	80	23.4 ± 6.5
"	"	10.0	80	11.6 ± 4.3
"	"	20.0	100	0.5 ± 0.7
"	Control	—	100	26.8 ± 8.6

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APPENDIX E
STATISTICAL ANALYSIS OF AMES TEST DATA

Table E-1. Slopes (revertants/mg), standard deviations of slopes, and degrees of freedom for Ames mutagenicity test (-S9) using extracts TA 98 and TA 100 for static pile composts.

	TA 98		TA 100	
% Soil	Day 0	Day 90	Day 0	Day 90
7%	83.2	9.8	204.8	2.1
	12.5	0.6	5.8	0.6
	18	20	8	10
10%	87.2	14.3	100.1	12.8
	5.4	0.5	2.8	1.1
	18	18	18	18
20%	309.5	21.6	546.4	14.2
	30.7	0.4	25.2	1.1
	18	20	18	10
30%	215.6	51.9	350.0	33.1
	16.1	3.7	25.0	1.0
	18	20	18	10
40%	160.1	86.9	286.1	64.8
	9.5	4.3	19.3	2.0
	18	20	18	10
100%	283.6	.	259.1	.
	10.7	.	20.4	.
	8	.	8	.

<u>Comparison of Ames Test slopes with 100% soil</u>

<u>Day = 0</u>

Obs	Soil Type	Percent	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
1	TA_98	7	68.49	70.66	72.84	45.65	1.72	2.52	20.92
2	TA_98	10	68.02	69.25	70.48	54.72	1.79	2.70	11.35
3	TA_98	20	-14.80	-9.15	-3.51	-3.39	1.71	2.48	26.10
4	TA_98	30	20.75	23.96	27.18	13.75	1.71	2.48	25.50
5	TA_98	40	41.41	43.54	45.67	30.93	1.74	2.58	16.29

Table E-1 (continued)

Day = 0

Obs	Soil Type	Percent	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
6	TA_100	7	16.35	20.98	25.61	8.11	1.80	2.74	10.44
7	TA_100	10	59.14	61.37	63.60	24.55	1.83	2.81	9.16
8	TA_100	20	-122.66	-110.88	-99.09	-33.55	1.72	2.51	21.93
9	TA_100	30	-43.37	-35.08	-26.80	-10.66	1.72	2.51	21.75
10	TA_100	40	-17.18	-10.40	-3.63	-3.47	1.74	2.56	17.26

Day = 90

Obs	Soil Type	Percent	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
1	TA_98	7	96.40	96.54	96.68	80.91	1.83	2.82	9.03
2	TA_98	10	94.79	94.96	95.13	79.60	1.83	2.82	9.02
3	TA_98	20	92.16	92.38	92.59	77.46	1.83	2.82	9.01
4	TA_98	30	80.93	81.71	82.50	66.75	1.81	2.76	9.99
5	TA_98	40	68.26	69.34	70.42	56.13	1.81	2.75	10.35
6	TA_100	7	99.04	99.19	99.33	39.84	1.83	2.82	9.01
7	TA_100	10	94.72	95.07	95.43	38.17	1.83	2.82	9.03
8	TA_100	20	94.09	94.50	94.92	37.93	1.83	2.82	9.04
9	TA_100	30	86.47	87.23	88.00	35.02	1.83	2.82	9.04
10	TA_100	40	73.51	75.00	76.50	30.01	1.83	2.81	9.15

Table E-2. Slopes (revertants/mg), standard deviations of slopes, and degrees of freedom for Ames mutagenicity test (-S9) using compost MC-3 and MC-4 for static pile compost.

	TA 98		TA 100	
Day	MC-3	MC-4	MC-3	MC-4
0	343.9 24.4 8	456.2 21.2 8	142.8 13.2 8	169.9 22.5 18
10	87.0 14.5 8	77.5 7.5 8	44.2 6.3 8	89.4 18.7 18
20	18.1 1.7 8	67.7 6.6 8	16.2 4.9 8	63.9 7.7 18
44	9.8 0.7 8	71.8 4.6 8	3.2 7.2 8	52.6 3.7 18

Comparison of Ames Test slopes with 100% soil.

The percent difference values are calculated using the following statistics for (100% soil - Day 0 values):

TA-098: Slope = 283.55 rev/mg
St. Dev. of Slope = 10.69
df = 8

TA-100: Slope = 259.10 rev/mg
St. Dev. of Slope = 20.39
df = 8

MC-3

Obs	Soil Type	Day	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
1	TA 98	0	-27.98	-21.28	-14.59	-7.17	1.78	2.67	12.33
2	TA 98	10	65.83	69.33	72.83	34.52	1.74	2.57	16.56
3	TA 98	20	93.16	93.61	94.07	77.57	1.82	2.79	9.44
4	TA 98	44	96.37	96.56	96.75	80.84	1.83	2.82	9.07
5	TA 100	0	40.38	44.88	49.39	15.13	1.75	2.59	15.44
6	TA 100	10	81.02	82.96	84.90	31.85	1.80	2.73	10.70
7	TA 100	20	92.38	93.75	95.11	36.64	1.81	2.76	10.02
8	TA 100	44	96.83	98.76	100.70	37.42	1.79	2.71	11.21

MC-4

9	TA 98	0	-67.46	-60.89	-54.32	-22.97	1.77	2.64	13.29
10	TA 98	10	70.76	72.65	74.55	49.95	1.75	2.58	16.10
11	TA 98	20	74.44	76.13	77.82	54.24	1.75	2.60	15.04
12	TA 98	44	73.38	74.66	75.95	57.59	1.78	2.67	12.18
13	TA 100	0	29.12	34.41	39.70	10.91	1.73	2.53	19.80
14	TA 100	10	61.64	65.50	69.36	22.08	1.74	2.57	16.77
15	TA 100	20	73.33	75.33	77.34	29.26	1.81	2.75	10.29
16	TA 100	44	78.35	79.70	81.04	31.76	1.83	2.80	9.30

Table E-3. Slopes (revertants/mg), standard deviations of slopes, and degrees of freedom for Ames mutagenicity test (+S9) using extracts TA 98 and TA 100 for static pile compost.

	TA 98		TA 100	
% Soil	Day 0	Day 90	Day 0	Day 90
7%	16.5	2.3	.	3.9
	2.0	0.3	.	0.9
	18	10	.	10
10%	20.6	3.8	31.9	6.7
	1.4	0.4	2.0	0.9
	8	10	8	10
20%	74.7	-0.1	194.3	[illegible]
	6.1	0.3	12.4	1.0
	8	6	8	6
30%	49.3	10.0	157.3	13.3
	2.5	0.5	16.8	1.6
	8	10	8	10
40%	38.9	23.5	98.8	38.5
	2.4	0.4	6.7	1.2
	8	10	8	10
100%	56.9	.	163.2	.
	3.3	.	7.2	.
	8	.	8	.

Comparison of Ames Test slopes with 100% soil.
Day = 0

Obs	Soil Type	Percent	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
1	TA 98	7	69.00	71.07	73.15	35.87	1.77	2.66	12.64
2	TA 98	10	61.70	63.88	66.07	32.51	1.78	2.68	12.08
3	TA 98	20	-40.16	-31.28	-22.41	-8.16	1.76	2.63	13.78
4	TA 98	30	8.95	13.36	17.76	5.88	1.74	2.57	16.77
5	TA 98	40	27.90	31.72	35.54	14.16	1.74	2.58	16.44
6	TA 100	10	79.46	80.47	81.47	55.75	1.81	2.75	10.21
7	TA 100	20	-25.32	-19.06	-12.79	-6.84	1.76	2.61	14.43
8	TA 100	30	-4.05	3.62	11.28	1.02	1.78	2.67	12.21
9	TA 100	40	36.19	39.46	42.73	20.62	1.73	2.55	17.92

Day = 90

Obs	Soil Type	Percent	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
10	TA 98	7	95.59	95.96	96.33	52.79	1.83	2.81	9.12
11	TA 98	10	92.79	93.30	93.82	51.21	1.83	2.81	9.20
12	TA 98	20	100.57	100.11	99.64	54.90	1.83	2.81	9.23
13	TA 98	30	81.58	82.50	83.41	45.09	1.83	2.80	9.35
14	TA 98	40	56.90	58.66	60.43	32.13	1.83	2.80	9.29
15	TA 98	7	92.04	93.09	94.15	49.82	1.81	2.76	10.15
16	TA 100	10	95.50	95.88	96.26	68.19	1.83	2.81	9.23
17	TA 100	20	98.53	99.01	99.50	70.04	1.82	2.79	9.43
18	TA 100	30	91.20	91.86	92.53	64.51	1.82	2.78	9.70
19	TA 100	40	75.49	76.38	77.27	54.02	1.82	2.79	9.44

Table E-4. Slopes (revertants/mg), standard deviations of slopes, and degrees of freedom for Ames mutagenicity test (+S9) using compost MC-3 and MC-4 for static pile compost.

	TA 98		TA 100	
Day	MC-3	MC-4	MC-3	MC-4
0	62.7 3.2 9	71.7 3.2 8	74.9 5.0 9	115.3 10.6 9
10	14.0 1.1 9	15.5 2.4 9	41.7 3.7 7	32.9 4.7 9
20	3.4 1.0 9	11.3 2.5 9	18.1 5.4 9	28.4 2.8 9
44	0.9 0.9 9	12.7 2.9 7	15.5 2.9 9	26.4 3.9 7

Comparison of Ames Test slopes with 100% soil.

The percent difference values are calculated using the following statistics for (100% soil - Day 0 values):

TA-098: Slope = 56.90 rev/mg
St. Dev. of slope = 3.26
df = 8

TA-100: Slope = 163.20 rev/mg
St. Dev. of Slope = 7.21
df = 8

MC-3

Obs	Soil Type	Day	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
1	TA 98	0	-15.59	-10.12	-4.65	-4.09	1.73	2.54	18.71
2	TA 98	10	73.79	75.41	77.04	39.59	1.80	2.72	10.89
3	TA 98	20	92.84	93.99	95.14	49.95	1.81	2.74	10.42
4	TA 98	44	97.37	98.45	99.54	52.47	1.81	2.75	10.30
5	TA 100	0	51.75	54.12	56.49	32.41	1.75	2.59	15.76
6	TA 100	10	72.64	74.42	76.21	46.94	1.76	2.63	13.65
7	TA 100	20	86.82	88.94	91.06	51.91	1.74	2.57	16.57
8	TA 100	44	89.30	90.51	91.73	60.41	1.79	2.69	11.67

Table E-4 (continued)

MC-4

Obs	Soil Type	Day	Lower 95% CI	%Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
9	TA 98	0	-32.04	-25.99	-19.94	-10.30	1.73	2.55	17.98
10	TA 98	10	69.90	72.81	75.73	32.76	1.74	2.57	16.58
11	TA 98	20	77.19	80.12	83.05	35.55	1.74	2.57	16.97
12	TA 98	44	73.98	77.64	81.31	31.36	1.74	2.57	17.00
13	TA 100	0	24.75	29.37	33.99	12.20	1.74	2.56	17.67
14	TA 100	10	77.94	79.87	81.80	48.64	1.75	2.60	15.19
15	TA 100	20	81.33	82.59	83.85	55.37	1.79	2.70	11.49
16	TA 100	44	82.06	83.83	85.60	52.19	1.76	2.62	14.08

Table E-5. Slopes (revertants/mg), standard deviations of slopes, and degrees of freedom for Ames mutagenicity test (-S9) using 10% soil compost for static pile compost.

Day	10% Soil TA 098	10% Soil TA 100
10	109.86	56.32
	9.20	4.97
	18	8
20	97.5	112.05
	6.75	4.92
	18	8
44	38.01	27.39
	5.40	4.38
	28	18

<u>Comparison of Ames Test slopes with 100% soil.</u>

Obs	Soil Type	Day	Lower 95% CI	% Diff	Upper 95% CI	T-Statistic	5% Level	1% Level	DF
1	TA_98	10	59.43	61.26	63.08	43.89	1.75	2.59	15.87
2	TA_98	20	64.16	65.61	67.07	50.25	1.77	2.66	12.71
3	TA_98	44	85.75	86.59	87.44	69.73	1.80	2.74	10.57
4	TA_100	10	76.45	78.26	80.07	30.55	1.81	2.76	10.07
5	TA_100	20	54.01	56.75	59.50	22.17	1.81	2.76	10.04
6	TA_100	44	88.39	89.43	90.46	35.53	1.82	2.80	9.42

Table E-6. Revertants per plate of compost extracts for Ames mutagenicity test (-S9) with strains TA 98 and TA 100.

Day = 0

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	7	0	0	24	24	29	29
2	TA 098	7	10	2	208	149	243	272
3	TA 098	7	20	4	273	271	524	449
4	TA 098	7	30	6	386	338	701	751
5	TA 098	7	40	8	423	465	991	902
6	TA 098	10	0	0	20	20	28	28
7	TA 098	10	10	2	394	403	391	425
8	TA 098	10	20	4	661	652	502	655
9	TA 098	10	30	6	.	.	728	771
10	TA 098	10	40	8	906	1014	880	920
11	TA 098	10	80	16	1468	1418	.	.
12	TA 098	20	0	0	25	25	39	39
13	TA 098	20	5	1	295	296	498	461
14	TA 098	20	10	2	640	634	810	790
15	TA 098	20	15	3	643	469	1016	1174
16	TA 098	20	20	4	1112	1204	1540	1586
17	TA 098	30	0	0	39	39	37	37
18	TA 098	30	5	1	295	296	403	354
19	TA 098	30	10	2	518	465	600	534
20	TA 098	30	15	3	643	469	862	890
21	TA 098	30	20	4	842	828	1048	988
22	TA 098	40	0	0	39	39	33	33
23	TA 098	40	5	1	207	252	284	268
24	TA 098	40	10	2	315	306	412	436
25	TA 098	40	15	3	456	502	578	686
26	TA 098	40	20	4	720	604	701	715
27	TA 098	100	0	0	37	37	.	.
28	TA 098	100	5	1	373	414	.	.
29	TA 098	100	10	2	606	600	.	.
30	TA 098	100	15	3	880	834	.	.
31	TA 098	100	20	4	1254	1192	.	.

Table E-6 (continued)

Day = 0

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
32	TA 100	7	0	0	134	134	.	.
33	TA 100	7	5	1	300	345	.	.
34	TA 100	7	10	2	514	546	.	.
35	TA 100	7	15	3	700	760	.	.
36	TA 100	7	20	4	980	928	.	.
37	TA 100	10	0	0	98	98	112	112
38	TA 100	10	10	2	350	334	318	323
39	TA 100	10	20	4	520	479	411	474
40	TA 100	10	30	6	.	.	653	706
41	TA 100	10	40	8	760	810	845	861
42	TA 100	10	80	16	1800	1728	.	.
43	TA 100	20	0	0	165	165	178	178
44	TA 100	20	5	1	780	808	680	725
45	TA 100	20	10	2	1134	1132	1320	1320
46	TA 100	20	15	3	2012	2020	1776	1876
47	TA 100	20	20	4	1864	2464	2604	2336
48	TA 100	30	0	0	165	165	134	134
49	TA 100	30	5	1	550	626	533	525
50	TA 100	30	10	2	740	784	830	950
51	TA 100	30	15	3	1226	640	1212	1320
52	TA 100	30	20	4	1768	1466	1662	1620
53	TA 100	40	0	0	165	165	163	163
54	TA 100	40	5	1	443	491	433	415
55	TA 100	40	10	2	804	892	750	694
56	TA 100	40	15	3	1012	1090	809	919
57	TA 100	40	20	4	1612	1464	1150	1127
58	TA 100	100	0	0	134	134	.	.
59	TA 100	100	5	1	414	432	.	.
60	TA 100	100	10	2	818	758	.	.
61	TA 100	100	15	3	982	986	.	.
62	TA 100	100	20	4	1020	1278	.	.

Table E-7. Revertants per plate of compost extracts for Ames mutagenicity test (-S9) with strains TA 98 and TA 100.

Day = 90

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	7	0	0	23	23	20	20
2	TA 098	7	5	1	.	.	24	32
3	TA 098	7	10	2	36	40	35	43
4	TA 098	7	20	4	40	44	74	74
5	TA 098	7	30	6	49	55	.	.
6	TA 098	7	40	8	80	92	101	97
7	TA 098	7	80	16	.	.	168	200
8	TA 098	10	0	0	20	20	20	20
9	TA 098	10	5	1	26	35	.	.
10	TA 098	10	10	2	65	46	56	48
11	TA 098	10	20	4	93	80	87	85
12	TA 098	10	40	8	125	101	138	144
13	TA 098	10	80	16	250	260	.	.
14	TA 098	20	0	0	23	23	23	23
15	TA 098	20	5	1	39	32	.	.
16	TA 098	20	10	2	67	67	64	68
17	TA 098	20	20	4	96	101	97	100
18	TA 098	20	30	6	.	.	139	149
19	TA 098	20	40	8	205	198	178	202
20	TA 098	20	80	16	358	374	.	.
21	TA 098	30	0	0	26	26	23	23
22	TA 098	30	5	1	79	57	.	.
23	TA 098	30	10	2	130	117	106	91
24	TA 098	30	20	4	224	245	142	136
25	TA 098	30	30	6	.	.	181	183
26	TA 098	30	40	8	444	416	225	252
27	TA 098	30	80	16	919	919	.	.
28	TA 098	40	0	0	26	26	23	23
29	TA 098	40	5	1	140	123	.	.
30	TA 098	40	10	2	230	250	181	171
31	TA 098	40	20	4	447	468	304	304
32	TA 098	40	30	6	.	.	472	412
33	TA 098	40	40	8	783	825	537	478
34	TA 098	40	80	16	1489	1467	.	.

Table E-7 (continued)

Day = 90

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
35	TA 098	100	0	0	37	37	.	.
36	TA 098	100	5	1	373	414	.	.
37	TA 098	100	10	2	606	600	.	.
38	TA 098	100	15	3	880	834	.	.
39	TA 098	100	20	4	1254	1192	.	.
40	TA 100	7	0	0	120	120	.	.
41	TA 100	7	5	1	144	120	.	.
42	TA 100	7	10	2	147	131	.	.
43	TA 100	7	20	4	143	141	.	.
44	TA 100	7	40	8	144	152	.	.
45	TA 100	7	80	16	174	147	.	.
46	TA 100	10	0	0	120	120	125	125
47	TA 100	10	5	1	.	.	147	153
48	TA 100	10	10	2	186	200	206	179
49	TA 100	10	20	4	220	254	176	184
50	TA 100	10	40	8	260	273	234	249
51	TA 100	10	80	16	.	.	332	340
52	TA 100	20	0	0	175	175	.	.
53	TA 100	20	5	1	238	242	.	.
54	TA 100	20	10	2	238	249	.	.
55	TA 100	20	20	4	293	275	.	.
56	TA 100	20	40	8	324	328	.	.
57	TA 100	20	80	16	416	444	.	.
58	TA 100	30	0	0	120	120	.	.
59	TA 100	30	5	1	166	170	.	.
60	TA 100	30	10	2	219	235	.	.
61	TA 100	30	20	4	281	291	.	.
62	TA 100	30	40	8	388	374	.	.
63	TA 100	30	80	16	658	685	.	.

Table E-7 (continued)

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
64	TA 100	40	0	0	120	120	.	.
65	TA 100	40	5	1	198	204	.	.
66	TA 100	40	10	2	293	272	.	.
67	TA 100	40	20	4	439	480	.	.
68	TA 100	40	40	8	736	673	.	.
69	TA 100	40	80	16	1186	1141	.	.
70	TA 100	100	0	0	134	134	.	.
71	TA 100	100	5	1	414	432	.	.
72	TA 100	100	10	2	818	758	.	.
73	TA 100	100	15	3	982	986	.	.
74	TA 100	100	20	4	1020	1278	.	.

Table E-8. Revertants per plate of compost extracts for Ames mutagenicity test (-S9) with strains TA 98 and TA 100.

Compost = MC-3

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	0	0	0	20.0	20.0	.	.
2	TA 098	0	5	1	528.0	474.0	.	.
3	TA 098	0	10	2	718.0	778.0	.	.
4	TA 098	0	15	3	912.0	980.0	.	.
5	TA 098	0	20	4	1440.0	1594.0	.	.
6	TA 098	10	0	0	16.7	16.7	.	.
7	TA 098	10	5	1	132.0	144.0	.	.
8	TA 098	10	10	2	101.0	258.0	.	.
9	TA 098	10	15	3	300.0	398.0	.	.
10	TA 098	10	20	4	295.0	397.0	.	.
11	TA 098	20	0	0	16.7	16.7	.	.
12	TA 098	20	5	1	26.0	28.0	.	.
13	TA 098	20	10	2	43.0	50.0	.	.
14	TA 098	20	15	3	80.0	73.0	.	.
15	TA 098	20	20	4	74.0	91.0	.	.
16	TA 098	44	0	0	16.7	16.7	.	.
17	TA 098	44	5	1	31.0	33.0	.	.
18	TA 098	44	10	2	39.0	39.0	.	.
19	TA 098	44	15	3	49.0	49.0	.	.
20	TA 098	44	20	4	61.0	53.0	.	.
21	TA 100	0	0	0	132.7	132.7	.	.
22	TA 100	0	5	1	337.0	312.0	.	.
23	TA 100	0	10	2	428.0	428.0	.	.
24	TA 100	0	15	3	506.0	542.0	.	.
25	TA 100	0	20	4	840.0	654.0	.	.
26	TA 100	10	0	0	187.0	187.0	.	.
27	TA 100	10	5	1	206.0	230.0	.	.
28	TA 100	10	10	2	252.0	269.0	.	.
29	TA 100	10	15	3	303.0	354.0	.	.
30	TA 100	10	20	4	396.0	309.0	.	.

Table E-8 (continued)

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
31	TA 100	20	0	0	187.0	187.0	.	.
32	TA 100	20	5	1	187.0	217.0	.	.
33	TA 100	20	10	2	223.0	214.0	.	.
34	TA 100	20	15	3	280.0	260.0	.	.
35	TA 100	20	20	4	243.0	225.0	.	.
36	TA 100	44	0	0	187.0	187.0	.	.
37	TA 100	44	5	1	261.0	274.0	.	.
38	TA 100	44	10	2	232.0	216.0	.	.
39	TA 100	44	15	3	240.0	187.0	.	.
40	TA 100	44	20	4	236.0	224.0	.	.

Table E-9. Revertants per plate of compost extracts for Ames mutagenicity test (-S9) with strains TA 98 and TA 100.

Compost = MC-4

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	0	0	0	20.0	20.0	.	.
2	TA 098	0	5	1	664.0	738.0	.	.
3	TA 098	0	10	2	982.0	1032.0	.	.
4	TA 098	0	15	3	1560.0	1462.0	.	.
5	TA 098	0	20	4	1844.0	1948.0	.	.
6	TA 098	10	0	0	41.3	41.3	.	.
7	TA 098	10	5	1	126.0	130.0	.	.
8	TA 098	10	10	2	186.0	152.0	.	.
9	TA 098	10	15	3	303.0	243.0	.	.
10	TA 098	10	20	4	406.0	307.0	.	.
11	TA 098	20	0	0	41.3	41.3	.	.
12	TA 098	20	5	1	169.0	129.0	.	
13	TA 098	20	10	2	185.0	225.0	.	.
14	TA 098	20	15	3	264.0	209.0	.	.
15	TA 098	20	20	4	355.0	317.0	.	.
16	TA 098	44	0	0	41.3	41.3	.	.
17	TA 098	44	5	1	146.0	127.0	.	.
18	TA 098	44	10	2	207.0	223.0	.	.
19	TA 098	44	15	3	256.0	305.0	.	.
20	TA 098	44	20	4	338.0	319.0	.	.
21	TA 100	0	0	0	132.7	132.7	92.7	92.7
22	TA 100	0	5	1	508.0	446.0	364.0	239.0
23	TA 100	0	10	2	656.0	652.0	494.0	450.0
24	TA 100	0	15	3	868.0	874.0	650.0	524.0
25	TA 100	0	20	4	1002.0	960.0	600.0	608.0
26	TA 100	10	0	0	132.7	132.7	92.7	92.7
27	TA 100	10	5	1	315.0	305.0	135.0	123.0
28	TA 100	10	10	2	444.0	382.0	234.0	183.0
29	TA 100	10	15	3	530.0	482.0	230.0	279.0
30	TA 100	10	20	4	700.0	568.0	300.0	349.0
31	TA 100	20	0	0	132.7	132.7	92.7	92.7
32	TA 100	20	5	1	211.0	227.0	137.0	149.0

Table E-9 (continued)

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
33	TA 100	20	10	2	289.0	300.0	180.0	212.0
34	TA 100	20	15	3	362.0	368.0	272.0	264.0
35	TA 100	20	20	4	394.0	448.0	315.0	301.0
36	TA 100	44	0	0	132.7	132.7	92.7	92.7
37	TA 100	44	5	1	178.0	199.0	157.0	173.0
38	TA 100	44	10	2	235.0	229.0	247.0	207.0
39	TA 100	44	15	3	263.0	316.0	292.0	256.0
40	TA 100	44	20	4	332.0	360.0	276.0	325.0

Table E-10. Revertants per plate of compost extracts for Ames mutagenicity test (+S9) with strains TA 98 and TA 100.

Day = 0

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	7	0	0	24	24	29	29
2	TA 098	7	10	2	62	52	57	75
3	TA 098	7	20	4	75	71	103	100
4	TA 098	7	30	6	90	100	144	157
5	TA 098	7	40	8	122	114	214	192
6	TA 098	10	0	0	28	28	.	.
7	TA 098	10	10	2	74	72	.	.
8	TA 098	10	20	4	88	121	.	.
9	TA 098	10	30	6	132	144	.	.
10	TA 098	10	40	8	202	200	.	.
11	TA 098	20	0	0	39	39	.	.
12	TA 098	20	5	1	94	107	.	.
13	TA 098	20	10	2	175	145	.	.
14	TA 098	20	15	3	207	222	.	.
15	TA 098	20	20	4	350	361	.	.
16	TA 098	30	0	0	39	39	.	.
17	TA 098	30	5	1	70	76	.	.
18	TA 098	30	10	2	138	118	.	.
19	TA 098	30	15	3	165	179	.	.
20	TA 098	30	20	4	246	226	.	.
21	TA 098	40	0	0	39	39	.	.
22	TA 098	40	5	1	89	73	.	.
23	TA 098	40	10	2	98	106	.	.
24	TA 098	40	15	3	158	141	.	.
25	TA 098	40	20	4	192	206	.	.
26	TA 098	100	0	0	37	37	.	.
27	TA 098	100	5	1	86	68	.	.
28	TA 098	100	10	2	154	158	.	.
29	TA 098	100	15	3	173	203	.	.
30	TA 098	100	20	4	270	262	.	.

Table E-10 (continued)

Day = 0

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
31	TA 100	10	0	0	112	112	.	.
32	TA 100	10	10	2	181	150	.	.
33	TA 100	10	20	4	227	206	.	.
34	TA 100	10	30	6	310	280	.	.
35	TA 100	10	40	8	384	348	.	.
36	TA 100	20	0	0	178	178	.	.
37	TA 100	20	5	1	332	408	.	.
38	TA 100	20	10	2	52[illegible]	514	.	.
39	TA 100	20	15	3	638	788	.	.
40	TA 100	20	20	4	1016	940	.	.
41	TA 100	30	0	0	178	178	.	.
42	TA 100	30	5	1	323	503	.	.
43	TA 100	30	10	2	433	449	.	.
44	TA 100	30	15	3	623	597	.	.
45	TA 100	30	20	4	896	836	.	.
46	TA 100	40	0	0	178	178	.	.
47	TA 100	40	5	1	307	245	.	.
48	TA 100	40	10	2	379	362	.	.
49	TA 100	40	15	3	532	446	.	.
50	TA 100	40	20	4	544	587	.	.
51	TA 100	100	0	0	134	134	.	.
52	TA 100	100	5	1	326	356	.	.
53	TA 100	100	10	2	495	519	.	.
54	TA 100	100	15	3	678	688	.	.
55	TA 100	100	20	4	790	768	.	.

Table E-11. Revertants per plate of compost extracts for Ames mutagenicity test (+S9) with strains TA 98 and TA 100.

Day = 90

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	7	0	0	20	20	.	.
2	TA 098	7	5	1	27	36	.	.
3	TA 098	7	10	2	33	24	.	.
4	TA 098	7	20	4	36	26	.	.
5	TA 098	7	40	8	44	50	.	.
6	TA 098	7	80	16	56	63	.	.
7	TA 098	10	0	0	20	20	.	.
8	TA 098	10	5	1	[illegible]	24	.	.
9	TA 098	10	10	2	[illegible]	32	.	.
10	TA 098	10	20	4	38	34	.	.
11	TA 098	10	40	8	50	34	.	.
12	TA 098	10	[illegible]	16	94	75	.	.
13	TA 098	20	[illegible]	[illegible]	23	23	.	.
14	TA 098	20	20	4	29	22	.	.
15	TA 098	[illegible]	40	8	36	26	.	.
16	TA 098	20	80	16	24	20	.	.
17	TA 098	30	0	0	26	26	.	.
18	TA 098	30	5	1	34	41	.	.
19	TA 098	30	10	2	56	44	.	.
20	TA 098	30	20	4	50	68	.	.
21	TA 098	30	40	8	100	84	.	.
22	TA 098	30	80	16	187	193	.	.
23	TA 098	40	0	0	26	26	.	.
24	TA 098	40	5	1	49	54	.	.
25	TA 098	40	10	2	65	68	.	.
26	TA 098	40	20	4	118	114	.	.
27	TA 098	40	40	8	191	208	.	.
28	TA 098	40	80	16	396	413	.	.

Table E-11 (continued)

Day = 90

OBS	EXTRACT	% Soil	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
29	TA 100	7	0	0	120	120	.	.
30	TA 100	7	5	1	175	159	.	.
31	TA 100	7	10	2	140	176	.	.
32	TA 100	7	20	4	163	161	.	.
33	TA 100	7	40	8	175	186	.	.
34	TA 100	7	80	16	208	199	.	.
35	TA 100	10	0	0	122	122	.	.
36	TA 100	10	5	1	157	162	.	.
37	TA 100	10	10	2	188	163	.	.
38	TA 100	10	20	4	184	181	.	.
39	TA 100	10	40	8	192	184	.	.
40	TA 100	10	80	16	240	267	.	.
41	TA 100	20	0	0	175	175	.	.
42	TA 100	20	20	4	177	179	.	.
43	TA 100	20	40	8	227	196	.	.
44	TA 100	20	80	16	206	188	.	.
45	TA 100	30	0	0	120	120	.	.
46	TA 100	30	5	1	162	174	.	.
47	TA 100	30	10	2	174	181	.	.
48	TA 100	30	20	4	178	186	.	.
49	TA 100	30	40	8	305	245	.	.
50	TA 100	30	80	16	384	302	.	.
51	TA 100	40	0	0	120	120	.	.
52	TA 100	40	5	1	180	200	.	.
53	TA 100	40	10	2	233	247	.	.
54	TA 100	40	20	4	286	309	.	.
55	TA 100	40	40	8	487	411	.	.
56	TA 100	40	80	16	758	759	.	.

Table E-12. Revertants per plate of compost extracts for Ames mutagenicity test (+S9) with strains TA 98 and TA 100.

Compost = MC-3

OBS	EXTRACT	Day	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	0	0	0	43	43	38	.
2	TA 098	0	5	1	73	85	.	.
3	TA 098	0	10	2	134	136	.	.
4	TA 098	0	15	3	238	216	.	.
5	TA 098	0	20	4	286	287	.	.
6	TA 098	10	0	0	17	23	10	.
7	TA 098	10	5	1	44	35	.	.
8	TA 098	10	10	2	47	42	.	.
9	TA 098	10	15	3	59	60	.	.
10	TA 098	10	20	4	77	74	.	.
11	TA 098	20	0	0	17	23	10	.
12	TA 098	20	5	1	24	15	.	.
13	TA 098	20	10	2	16	22	.	.
14	TA 098	20	15	3	29	23	.	.
15	TA 098	20	20	4	31	31	.	.
16	TA 098	44	0	0	17	23	10	.
17	TA 098	44	5	1	18	23	.	.
18	TA 098	44	10	2	15	15	.	.
19	TA 098	44	15	3	23	16	.	.
20	TA 098	44	20	4	19	24	.	.
21	TA 100	0	0	0	80	104	89	.
22	TA 100	0	5	1	160	153	.	.
23	TA 100	0	10	2	216	210	.	.
24	TA 100	0	15	3	308	352	.	.
25	TA 100	0	20	4	350	413	.	.
26	TA 100	10	0	0	169	200	192	.
27	TA 100	10	5	1	252	240	.	.
28	TA 100	10	10	2	292	275	.	.
29	TA 100	10	20	4	337	374	.	.

Table E-12 (continued)

OBS	EXTRACT	Day	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
30	TA 100	20	0	0	169	200	192	.
31	TA 100	20	5	1	257	244	.	.
32	TA 100	20	10	2	260	247	.	.
33	TA 100	20	15	3	288	280	.	.
34	TA 100	20	20	4	248	253	.	.
35	TA 100	44	0	0	169	200	192	.
36	TA 100	44	5	1	212	220	.	.
37	TA 100	44	10	2	231	248	.	.
38	TA 100	44	15	3	248	251	.	.
39	TA 100	44	20	4	247	240	.	.

Table E-13. Revertants per plate of compost extracts for Ames mutagenicity test (+S9) with strains TA 98 and TA 100.

Compost = MC-4

OBS	EXTRACT	Day	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
1	TA 098	0	0	0	13	13	.	.
2	TA 098	0	20	4	215	206	.	.
3	TA 098	0	40	8	468	397	.	.
4	TA 098	0	80	16	1072	1066	.	.
5	TA 098	0	100	20	1360	1502	.	.
6	TA 098	10	0	0	43	43	38	.
7	TA 098	10	5	1	58	39	.	.
8	TA 098	10	10	2	60	79	.	.
9	TA 098	10	15	3	75	74	.	.
10	TA 098	10	20	4	92	123	.	.
11	TA 098	20	0	0	43	43	38	.
12	TA 098	20	5	1	41	33	.	.
13	TA 098	20	10	2	43	54	.	.
14	TA 098	20	15	3	50	58	.	.
15	TA 098	20	20	4	92	95	.	.
16	TA 098	44	0	0	43	43	38	.
17	TA 098	44	5	1	33	50	.	.
18	TA 098	44	10	2	59	52	.	.
19	TA 098	44	15	3	73	90	.	.
20	TA 100	0	0	0	80	104	89	.
21	TA 100	0	5	1	245	210	.	.
22	TA 100	0	10	2	321	298	.	.
23	TA 100	0	15	3	428	564	.	.
24	TA 100	0	20	4	479	579	.	.
25	TA 100	10	0	0	80	104	89	.
26	TA 100	10	5	1	111	121	.	.
27	TA 100	10	10	2	110	137	.	.
28	TA 100	10	15	3	215	152	.	.
29	TA 100	10	20	4	215	236	.	.
30	TA 100	20	0	0	80	104	89	.
31	TA 100	20	5	1	142	149	.	.
32	TA 100	20	10	2	166	145	.	.

Table E-13 (continued)

OBS	EXTRACT	Day	uL/Plate	Dose (mg)	Day 1 Rep1	Day 1 Rep2	Day 2 Rep1	Day 2 Rep2
33	TA 100	20	15	3	193	190	.	.
34	TA 100	20	20	4	213	198	.	.
35	TA 100	44	0	0	80	104	89	.
36	TA 100	44	5	1	133	116	.	.
37	TA 100	44	10	2	155	168	.	.
38	TA 100	44	15	3	157	172	.	.

Table E-14. Revertants per plate of compost extracts for Ames mutagenicity test (-S9) with strains TA 98 and TA 100.

10 % Soil

OBS	Extract	Day	uL/Plate	Dose(mg)	Day 1 Rep 1	Day 1 Rep 2	Day 2 Rep 1	Day 2 Rep 2	Day 3 Rep 1	Day 3 Rep 2
1	TA 098	10	0	0	40	40	19	19	.	.
2	TA 098	10	10	2	248	250	313	361	.	.
3	TA 098	10	20	4	424	410	576	623	.	.
4	TA 098	10	30	6	569	452	880	940	.	.
5	TA 098	10	40	8	860	820	1006	992	.	.
6	TA 098	20	0	0	40	40	28	28	.	.
7	TA 098	20	10	2	225	182	367	304	.	.
8	TA 098	20	20	4	358	355	560	530	.	.
9	TA 098	20	30	6	485	643	660	720	.	.
10	TA 098	20	40	8	755	709	907	950	.	.
11	TA 098	44	0	0	40	40	28	28	20	20
12	TA 098	44	10	2	76	82	109	91	107	117
13	TA 098	44	20	4	150	138	144	150	170	204
14	TA 098	44	30	6	164	176	208	212	280	331
15	TA 098	44	40	8	226	194	253	237	587	565
16	TA 100	10	0	0	173	173	.	.	.	.
17	TA 100	10	10	2	330	346	.	.	.	.
18	TA 100	10	20	4	496	458	.	.	.	.
19	TA 100	10	30	6	470	509	.	.	.	.
20	TA 100	10	40	8	686	635	.	.	.	.
21	TA 100	20	0	0	112	112	.	.	.	.
22	TA 100	20	10	2	388	356	.	.	.	.
23	TA 100	20	20	4	633	544	.	.	.	.
24	TA 100	20	30	6	724	770	.	.	.	.
25	TA 100	20	40	8	1014	1076	.	.	.	.
26	TA 100	44	0	0	112	112	.	.	.	.
27	TA 100	44	10	2	120	112	.	.	.	.
28	TA 100	44	20	4	170	166	.	.	.	.
29	TA 100	44	30	6	198	198	.	.	.	.
30	TA 100	44	40	8	242	218	.	.	.	.
31	TA 100	44	0	0	96	96	.	.	.	.
32	TA 100	44	10	2	133	154	.	.	.	.
33	TA 100	44	20	4	157	161	.	.	.	.
34	TA 100	44	30	6	248	262	.	.	.	.
35	TA 100	44	40	8	411	447	.	.	.	.

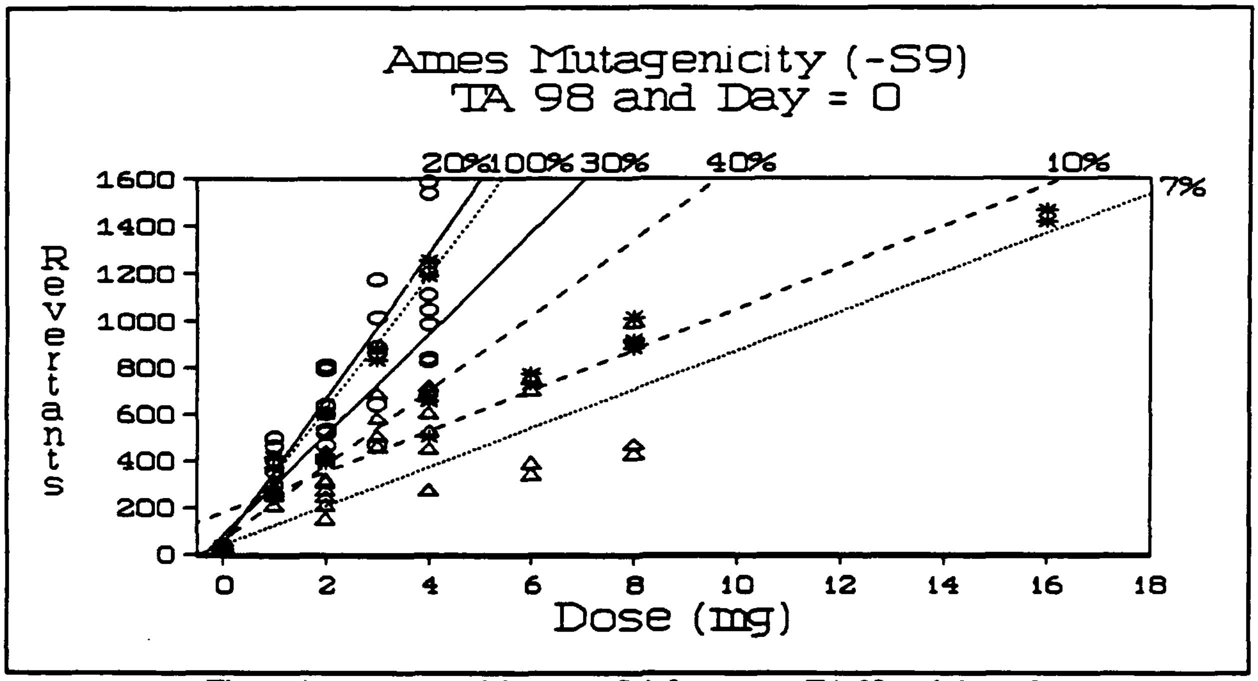

Fig. 1. Ames mutagenicity test (-S9) for extract TA 98 and day =0. Percentages of soil composition are indicated on the graph.

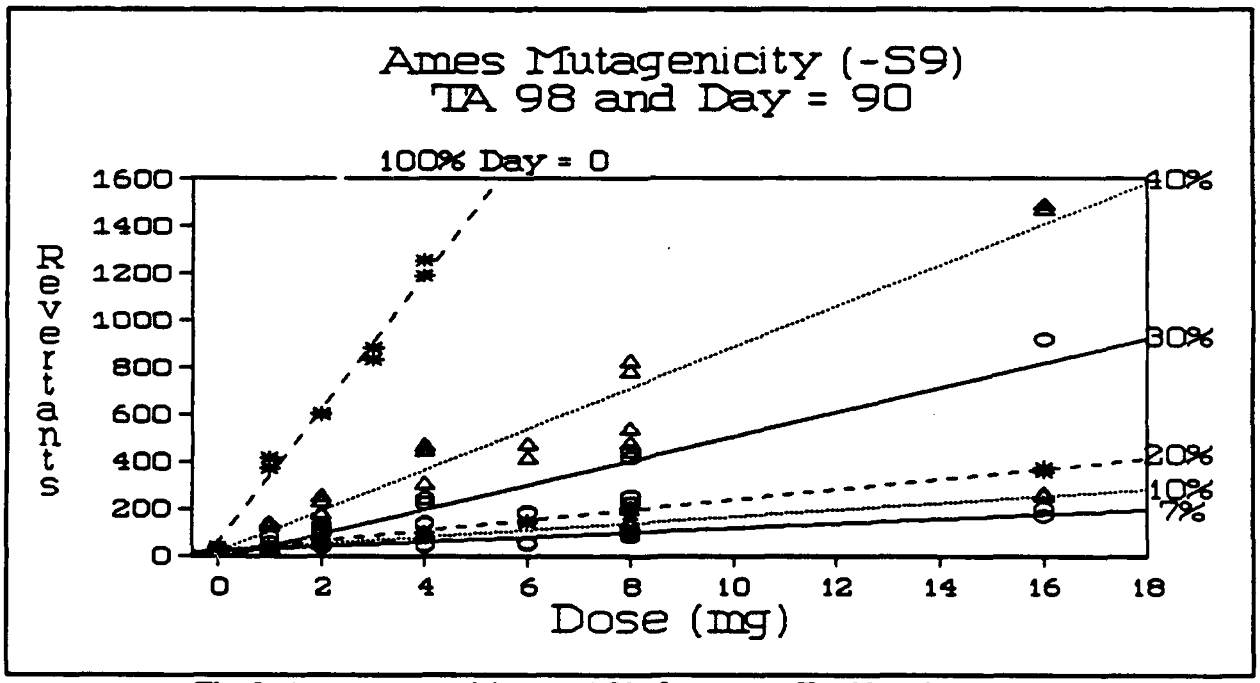

Fig. 2. Ames mutagenicity test (-S9) for extract TA 98 and day = 90. Percentages of soil composition are indicated on the graph.

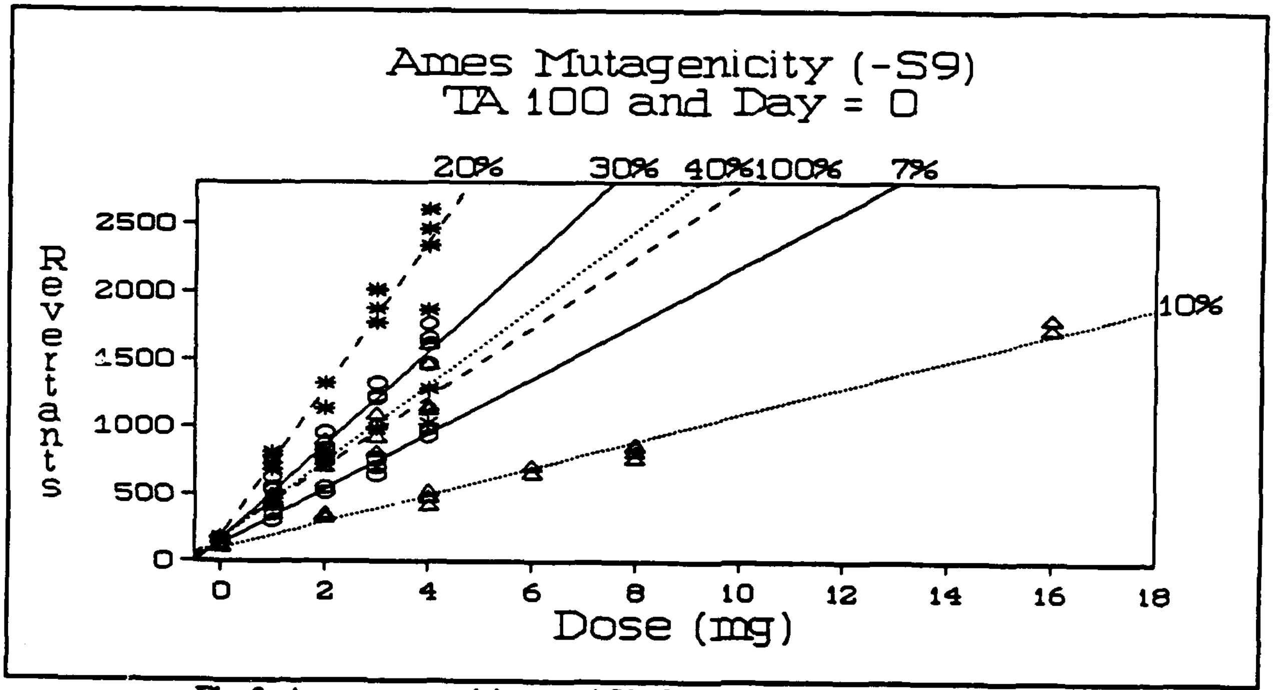

Fig. 3. Ames mutagenicity test (-S9) for extract TA 100 and day = 0. Percentages of soil composition are indicated on the graph.

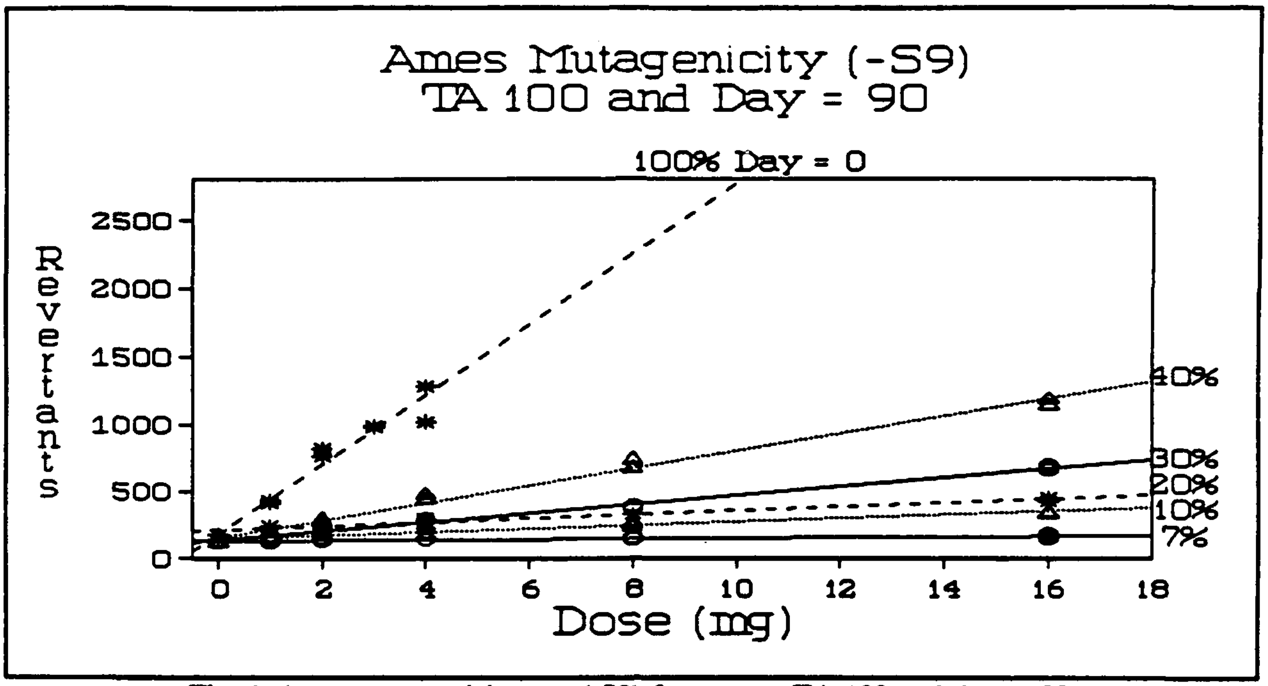

Fig. 4. Ames mutagenicity test (-S9) for extract TA 100 and day = 90. Percentages of soil composition are indicated on the graph.

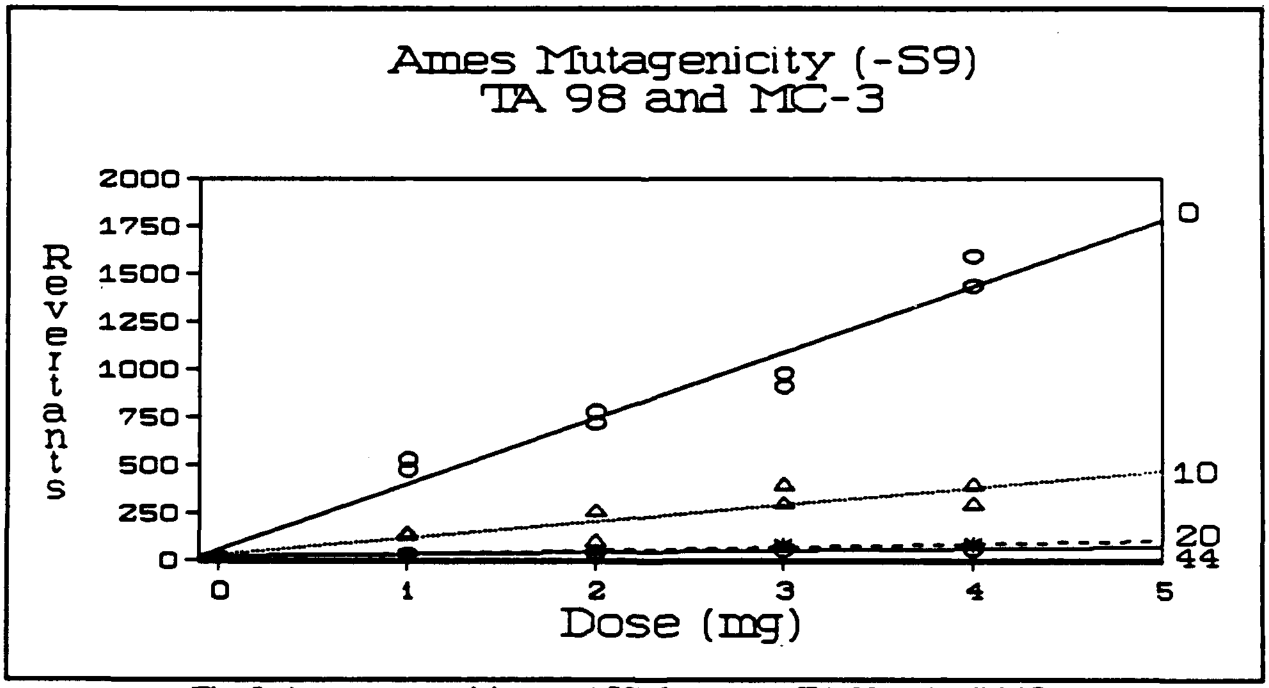

Fig. 5. Ames mutagenicity test (-S9) for extract TA 98 and soil MC-3. Lengths of test days are indicated on the graph.

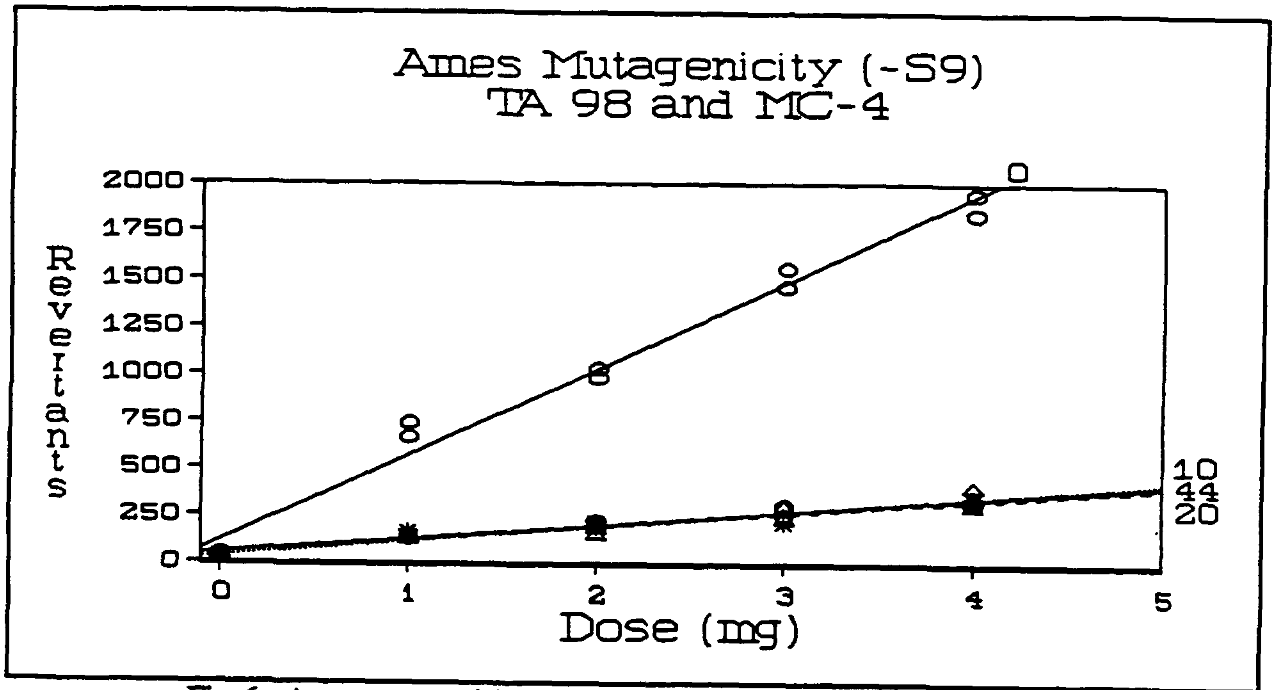

Fig. 6. Ames mutagenicity test (-S9) for extract TA 98 and soil MC-4. Lengths of test days are indicated on the graph.

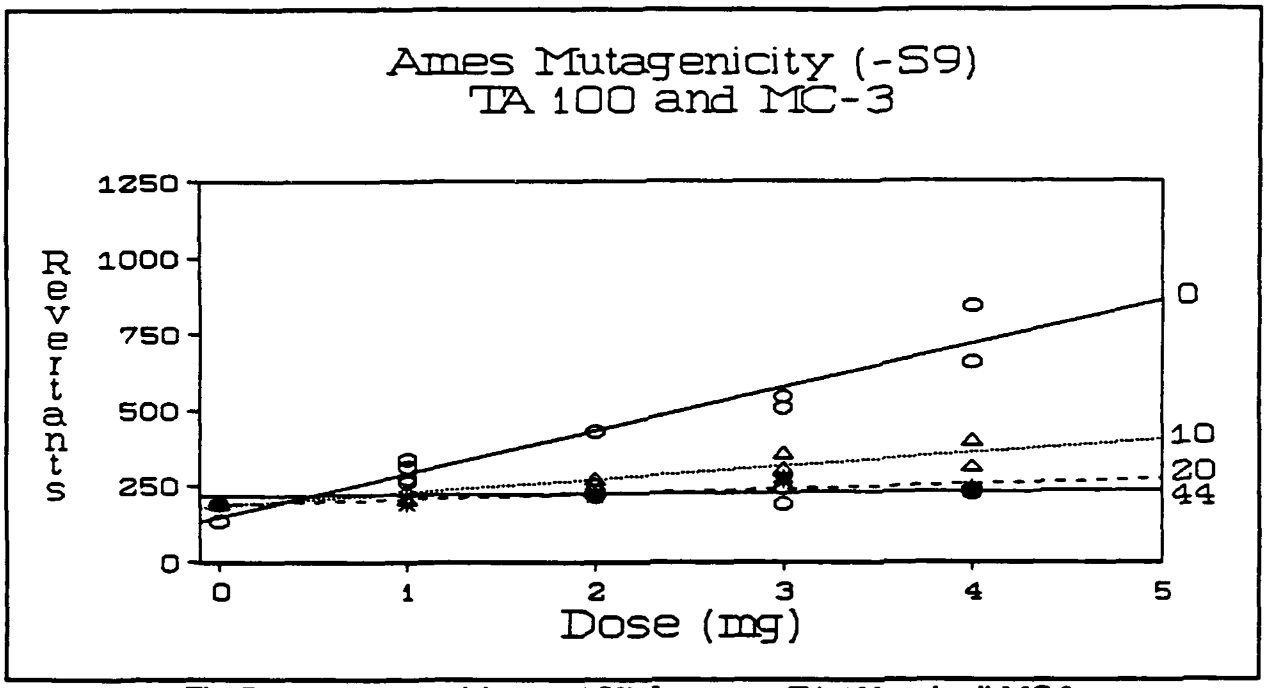

Fig. 7. Ames mutagenicity test (-S9) for extract TA 100 and soil MC-3. Lengths of test days are indicated on the graph.

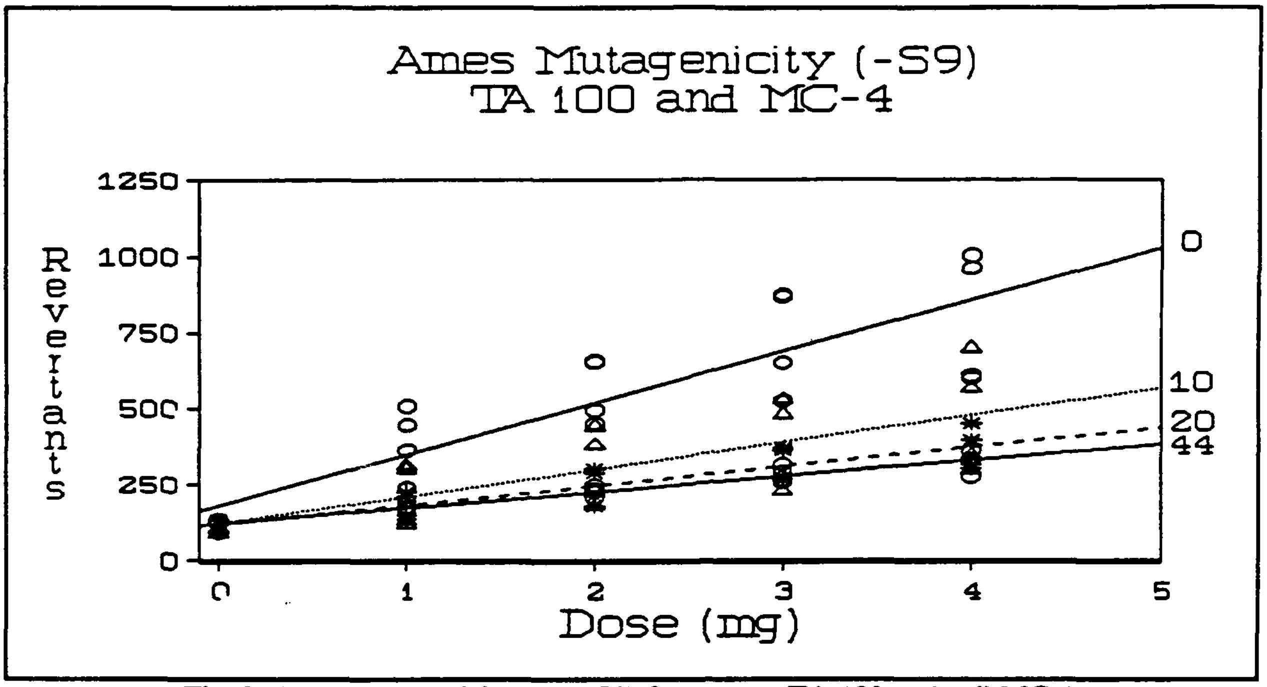

Fig. 8. Ames mutagenicity test (-S9) for extract TA 100 and soil MC-4. Lengths of test days are indicated on the graph.

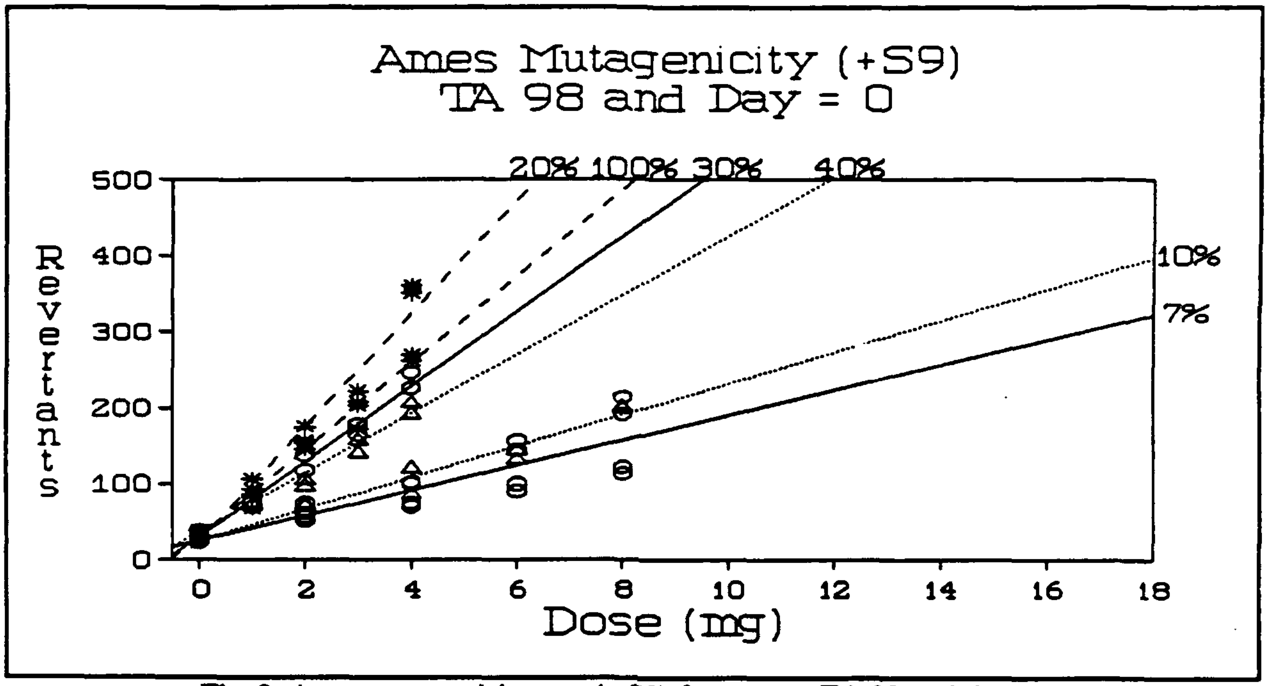

Fig. 9. Ames mutagenicity test (+S9) for extract TA 98 and day =0.
Percentages of soil composition are indicated on the graph.

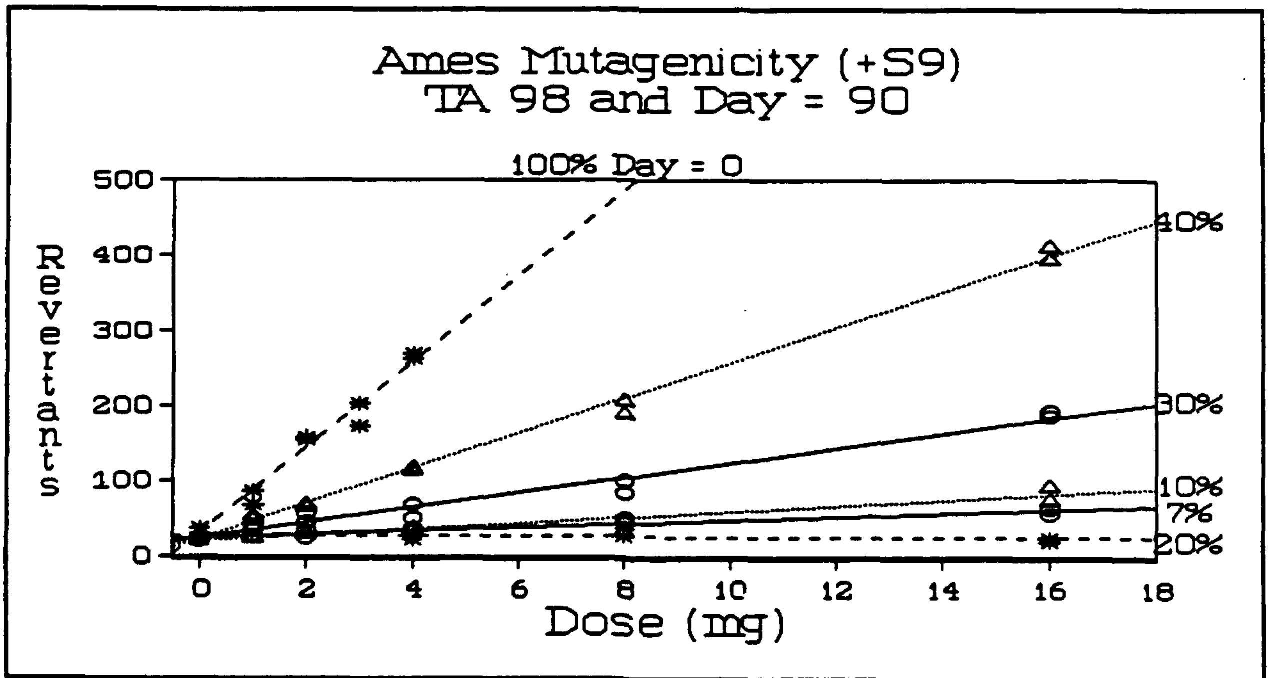

Fig. 10. Ames mutagenicity test (+S9) for extract TA 98 and day = 90.
Percentages of soil composition are indicated on the graph.

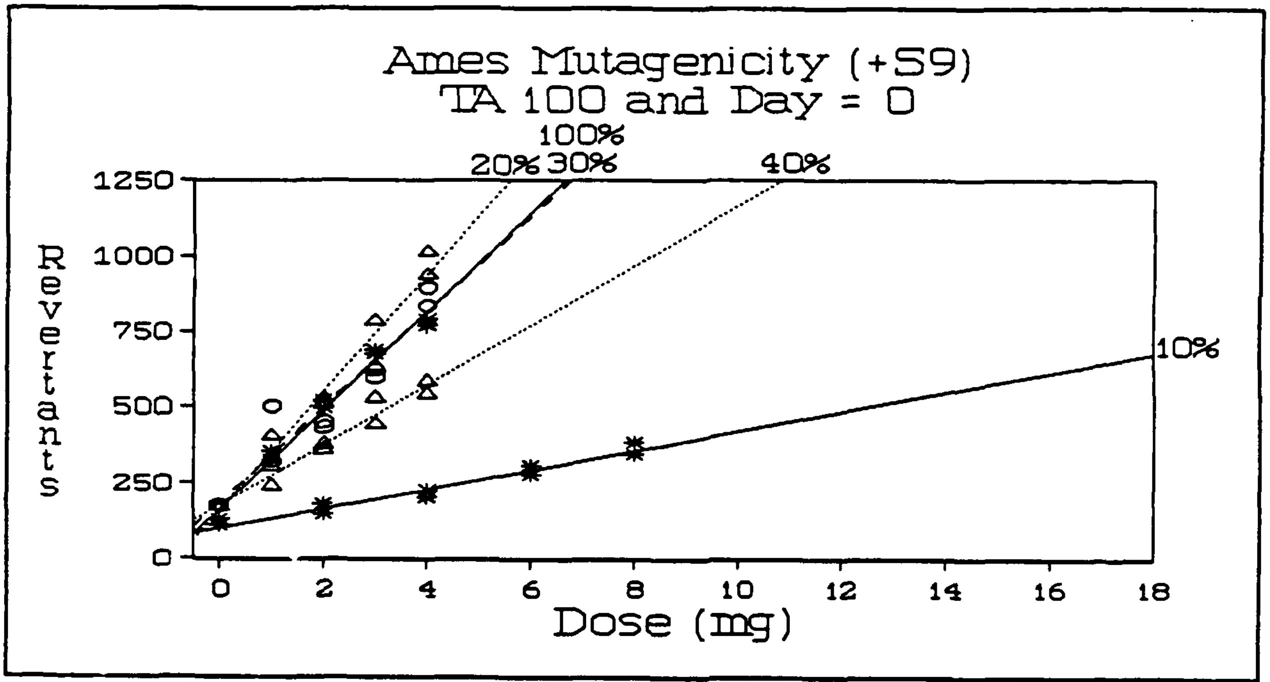

Fig. 11. Ames mutagenicity test (+S9) for extract TA 100 and day = 0. Percentages of soil composition are indicated on the graph.

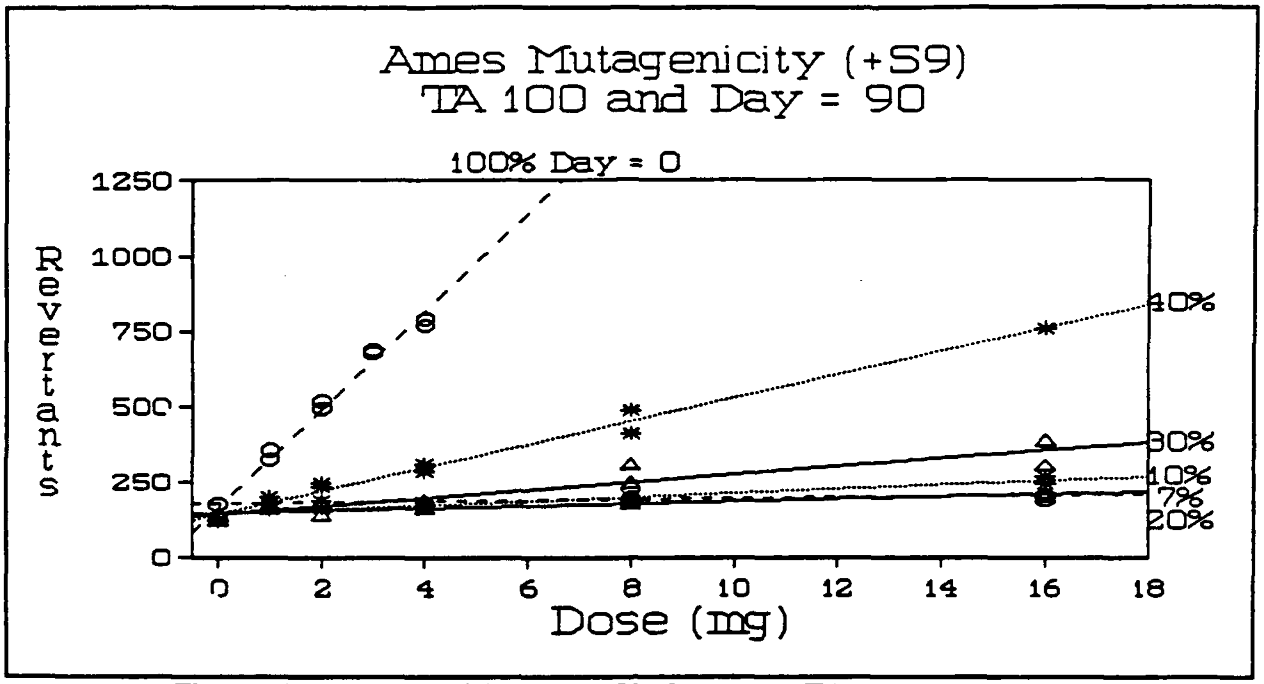

Fig. 12. Ames mutagenicity test (+S9) for extract TA 100 and day = 90. Percentages of soil composition are indicated on the graph.

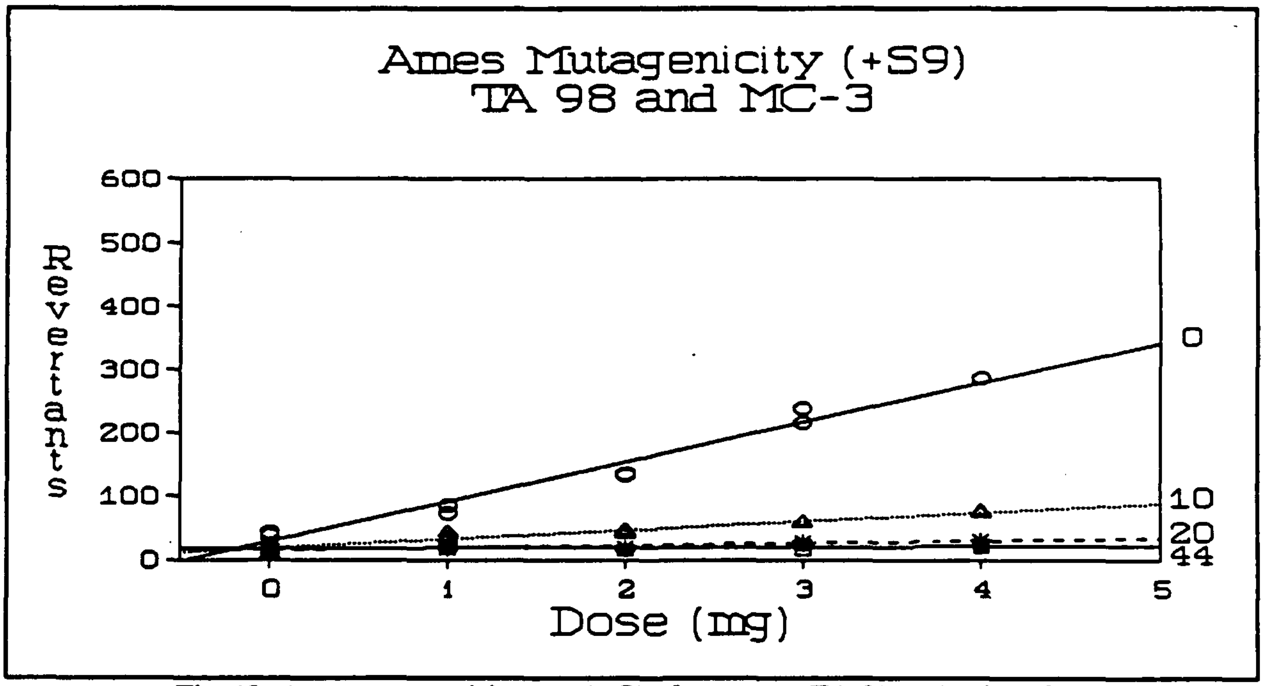

Fig. 13. Ames mutagenicity test (+S9) for extract TA 98 and soil MC-3. Lengths of test days are indicated on the graph.

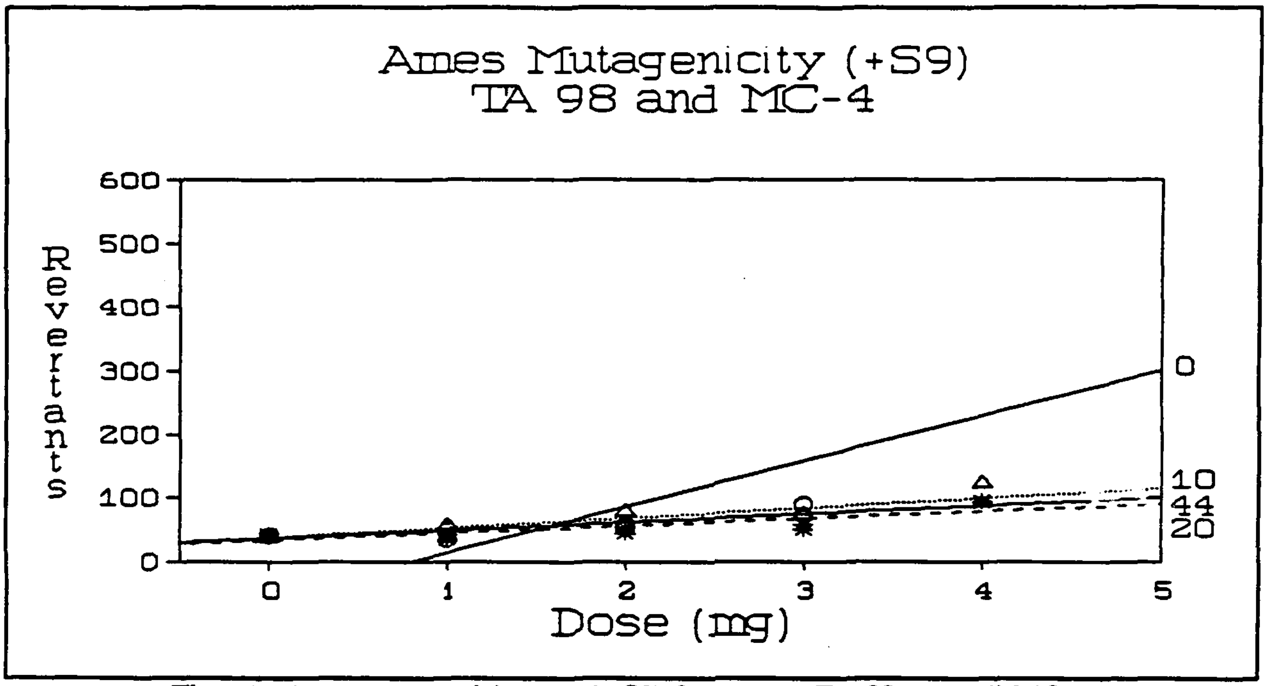

Fig. 14. Ames mutagenicity test (+S9) for extract TA 98 and soil MC-4.
Lengths of test days are indicated on the graph.

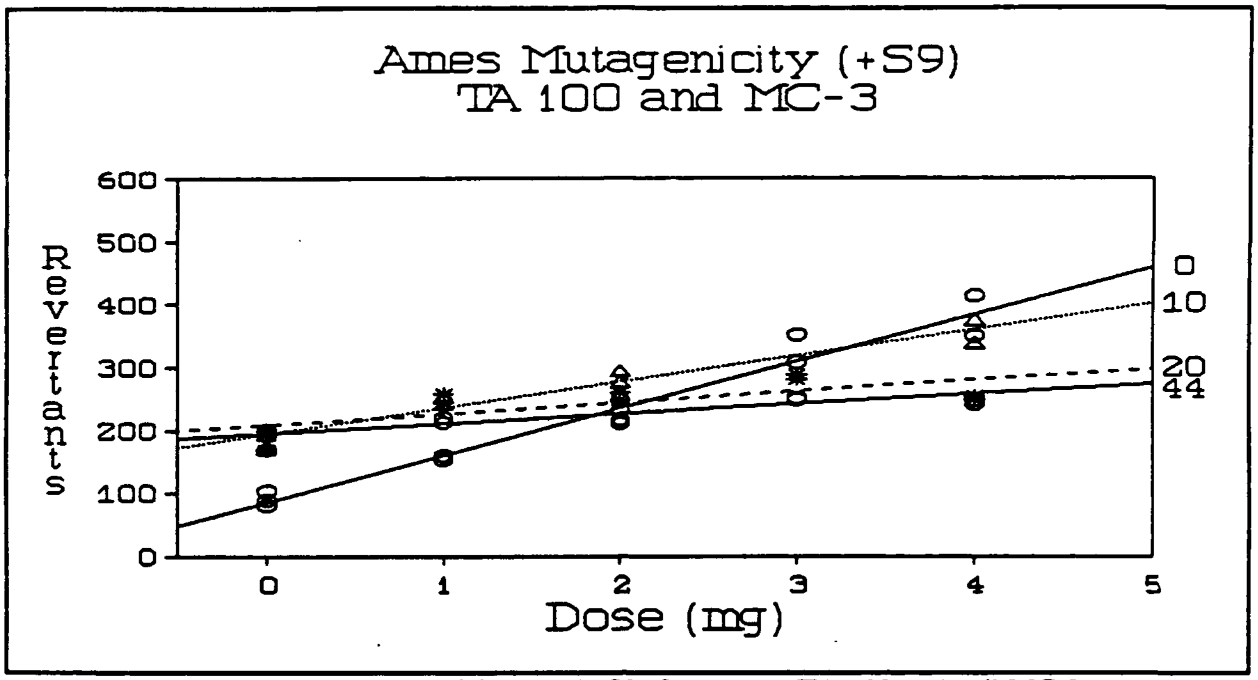

Fig. 15. Ames mutagenicity test (+S9) for extract TA 100 and soil MC-3. Lengths of test days are indicated on the graph.

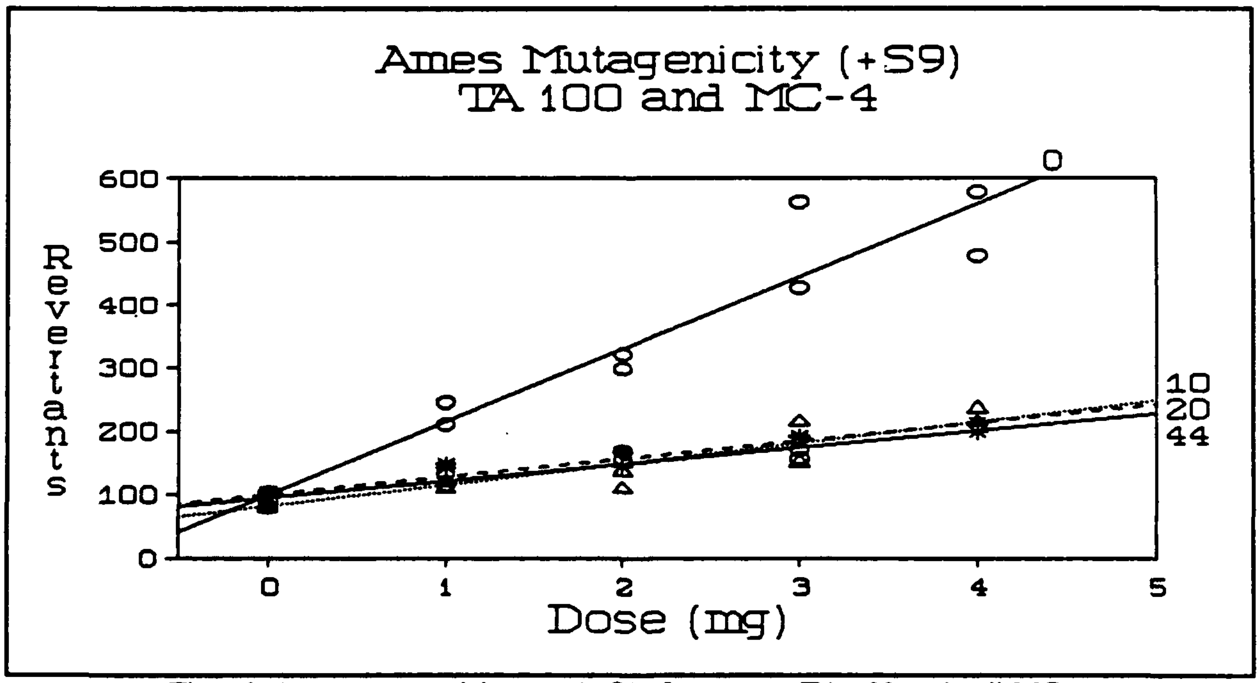

Fig. 16. Ames mutagenicity test (+S9) for extract TA 100 and soil MC-4. Lengths of test days are indicated on the graph.

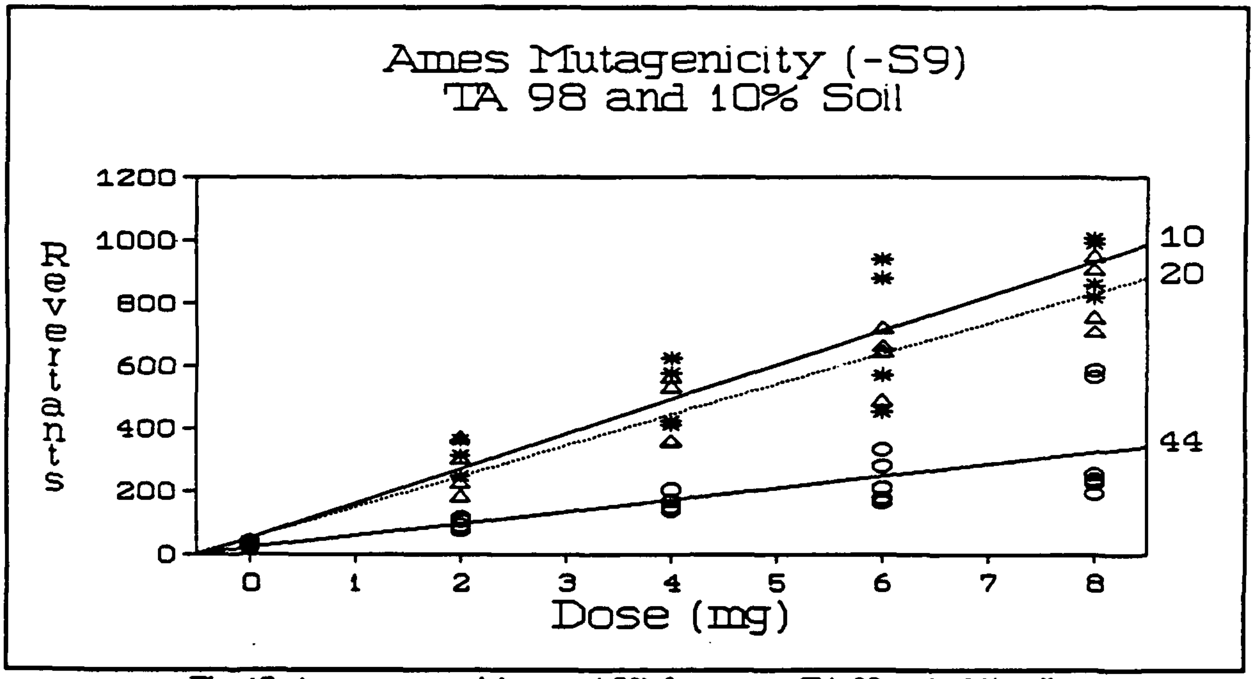

Fig. 17. Ames mutagenicity test (-S9) for extract TA 98 and 10% soil. Lengths of test days are indicated on the graph.

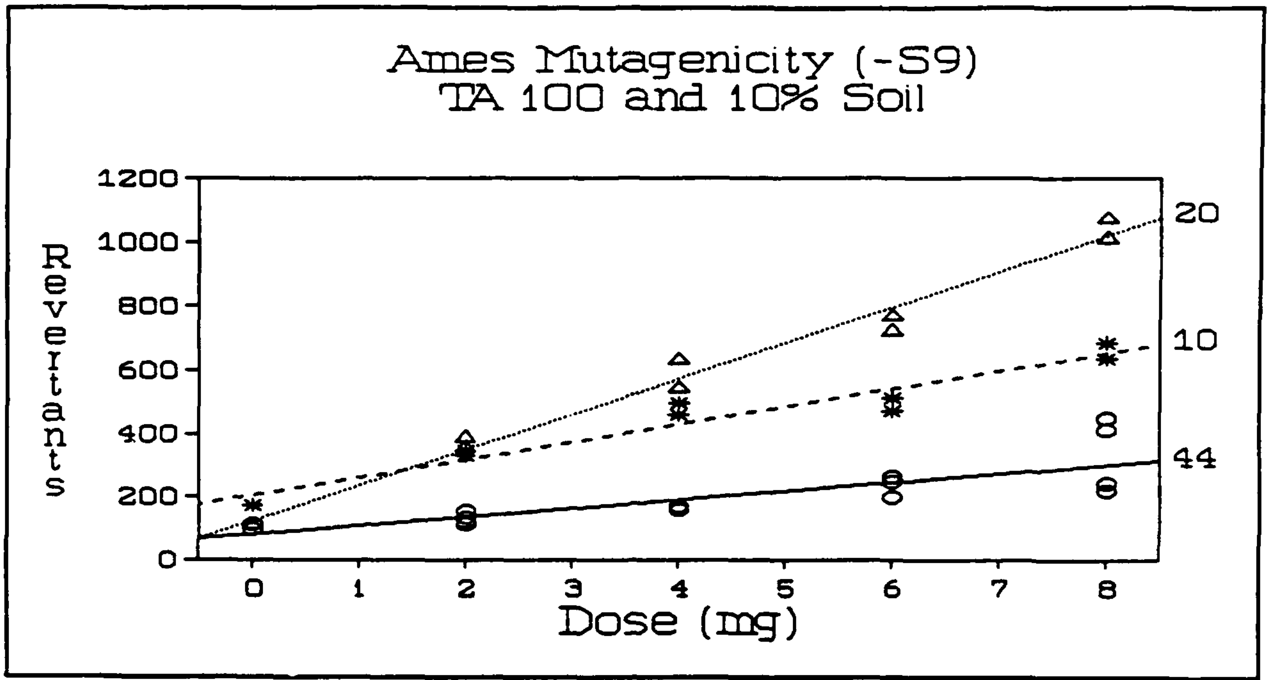

Fig. 18. Ames mutagenicity test (-S9) for extract TA 100 and 10% soil. Lengths of test days are indicated on the graph.

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